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STOCKHOLM SWEDEN

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1975, Nov 24–28 Int Symposium on Cervical Lymphonode Metastasis at Istituto Nazionale per lo Studio e la Cura dei Tumori, Via Venezian 1, Milan, Italy

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AN EVOKED RESPONSE STUDY OF THE FIRST-ORDER DIFFERENCE TONE IN MAN

J L Gorny[1] and R A Butler

From the Department of Surgery (Otolaryngology), University of Chicago, Chicago, Ill, USA

(Received August 13, 1974)

Abstract The amplitude of the N_1–P_2 component of a response evoked by a 250 Hz test stimulus presented once each 5 sec was measured Interposed at a rate of one per sec were *either* 1) a 4 000 Hz tone 2) a 4 000 Hz and a 4 250 Hz tone presented simultaneously which generated within the ear an audible 250 Hz difference tone, or 3) a 250 Hz tone The results indicated that the N_1–P_2 component when preceded by the 4 000 Hz plus 4 250 Hz stimulus pair was larger than when preceded by a 250 Hz tone, but smaller than when preceded by a 4 000 Hz tone Apparently the processing of the difference tone is unlike that of a sinusoid of the same frequency or that of a high frequency sinusoid which participates in the generation of the difference tone Parallel electrophysiological data from cochlear recordings are discussed

When two tones, differing in frequency, are presented simultaneously at the higher intensity levels, a multitude of additional tones called combination tones, can be detected under suitable conditions of observations One clearly audible combination tone is the first order difference tone whose tonal frequency corresponds to h–l where h and l are the frequencies of the higher and lower primary tones, respectively The question which motivated the present experiment relates to the processing of the first order difference tone Is it processed in the same manner as a sinusoid of the same frequency or as one member of the pair of primary tones which elicit the difference tone? We designed an evoked response experiment on humans as a means toward answering this question But first, a word about the human auditory evoked response as a suitable candidate for the task The most prominent feature of the auditory evoked response in the N_1–P_2 component, and its amplitude usually serves as the dependent variable when studying the influence of stimulus conditions on the electrical response of the brain Yet when examining the form of the response, one cannot deduce the auditory stimulus which evoked it For example, the response evoked by a first order difference tone will resemble that evoked by any other sound There is however, a way to get a handle on the problem Specifically N_1–P_2 amplitude to a sinusoid of one frequency will suffer a greater decrement if preceded by a stimulus of identical frequency than if preceded by a stimulus of some other frequency (Butler, 1968) In our experiment we simply preceded a 250 Hz test stimulus by either 1) another sinusoid of 250 Hz, or 2) a pair of primary tones (4 000 and 4 250 Hz) which generated a *first order* difference tone of 250 Hz, or 3) a 4 000 Hz tone which, of course, was one member of the tonal complex that elicited the difference tone We reasoned that if both the 250 Hz sinusoid and the difference tone have the same suppressive effect on the test stimulus, one could argue that they are processed in a similar manner If on the other hand, the influence of the difference tone mimicked that of the 4 000 Hz tone, then the inference would be that these two sets of stimuli are processed similarly

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[1] Present address Dept of Otolaryngology, Cook County Hospital, Chicago, Ill 60612 USA

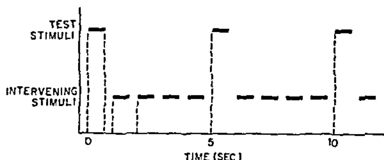


Fig 1 The temporal sequence of stim[illegible] presentation The test stimulus was alw[illegible] 250 Hz.

MAIN EXPERIMENT

Method

Fig 1 illustrates the sequence of stimulation The duration of all stimuli was 100 msec, their rise-fall time was 10 msec The test stimulus was presented once each 5 sec, the intervening stimuli were delivered once each sec between successive test stimuli Four conditions of intervening stimulation were established For Conds 1, 2, and 3, 4 000 Hz, 4 000 plus 4 250 Hz, and 250 Hz intervened, respectively In Cond 4, no auditory stimuli were interposed To preclude the possibility of the difference tone (Cond 2) being produced by the stimulus generating equipment and hence contained in the auditory input to the ear, the 4 000 Hz tone was generated and controlled by one audio channel and the 4 250 Hz was generated and controlled by a separate Furthermore, each tone activated a [illegible]fferent loudspeaker In fact, 3 loudspeakers were utilized—1 for the 4 000 Hz stimulus, 1 for the 4 250 Hz stimulus which was also used as the source of the 250 Hz intervening stimulus when appropriate (Cond 3), and the 3rd loudspeaker generated the 250 Hz test stimulus The loudspeakers, 4-inch diam, were housed in a 6½ × 6½ × 6 inch wooden cabinets These cabinets were bound together in a pyramidal pattern and fixed to a vertical post The listener was seated in a chair equipped with a headrest The chair was situated so that the listener's left ear corresponded in height to the center of the pyramid of loudspeakers Distance between the pyramid's center point and the listener s pinna was approximately 6 inch

Stimulus intensity for the 4 000 and 4 250 Hz primary tones was 80 dB SPL as measured at a position which, during testing, would have been occupied by the listener's left pinna At t[illegible] intensity, the loudness of the 250 Hz differen[illegible] tone was estimated by introducing an explori[illegible] tone of 253 Hz The intensity of the explori[illegible] tone which produced the best beats as agre[illegible] upon by 3 trained listeners was 45 dB SP[illegible] Hence, the intensity of the 250 Hz test stimul[illegible] was set at 45 dB SPL as was that of the 250 [illegible] intervening stimulus

Referring again to Fig 1, an experiment[illegible] condition consisted of a sequence of test stimuli [illegible] intervening stimuli which continued until t[illegible] test stimulus had been presented 75 times On[illegible] response evoked by the test stimuli were tran[illegible] mitted to the computer (FabriTek, Model 107[illegible] These were recorded between the vertex a[illegible] paired earlobes A head strap secured the vert[illegible] electrode, plastic clips secured the earlobe ele[illegible] trodes The waveform of the summed respons[illegible] was displayed on an oscilloscope screen and t[illegible] amplitude of the N_1–P_2 component was ca[illegible] culated (see Butler, 1972)

Eight persons, whose hearing acuity was wit[illegible] in 15 dB of audiometric zero (ISO Standard[illegible] 1964) for the frequencies 250–8 000 Hz, serve[illegible] as subjects A subject was given 4 test session[illegible] and each session consisted of the 4 experimenta[illegible] conditions A 4 × 4 Latin square governed th[illegible] order in which the experimental conditions wer[illegible] presented Rows represented subjects, column[illegible] represented ordinal position of tests within [illegible] session, and Latin letters represented the exper[illegible] mental conditions For each listener, the N_1 P_2 component for the various experimental cond[illegible] tions was summed over the 4 test sessions The[illegible] values, 1 for each experimental condition, we[illegible] then ordered for further statistical treatment

Before describing our results, it should b[illegible]

mentioned that the frequency of the test and intervening stimuli were monitored repeatedly during the course of a session by means of a Hewlett-Packard Electronic Counter (Model 5221A) We immediately corrected for any slight drift of the oscillators Testing was conducted in a sound treated room

Results

Fig 2 summarizes the data N_1–P_2 amplitude recorded under Cond 4 was taken as the normal value of a response to the 250 Hz test stimulus since no other auditory stimuli intervened to affect the amplitude adversely It is evident from this histogram that when a 250 Hz tone intervened (Cond 3), response amplitude to the test stimulus was reduced severely The 2 primaries which produced a 250 Hz difference tone failed to exert a comparable suppressive effect (Cond 2) Yet, they had a greater suppressive effect than did the presentation of only one of the primaries (Cond 1) Mean amplitudes of the N_1–P_2 component in μV were 4 5, 3 4, and 2 0 for Conds 1 through 3, respectively With no auditory stimuli intervening, mean response amplitude to the test stimulus was 5 6 μV (Cond 4) Not only were differences in response amplitudes among experimental conditions statistically significant ($p<0.01$) as indicated by a variance analysis, but each mean amplitude value listed above differed significantly from all the other mean values ($p<0.01$ "F" statistic) In brief, numerical differences between mean response amplitudes associated with the 4 experimental conditions were small, but highly consistent Each listener's data showed the same pattern as that which characterized the group data

Comment

Upon further reflection there appeared to be a problem inherent in the design of the experiment Specifically, how can one expect high frequency stimuli (Cond 2) to exert the same influence on the response to the test stimulus as exerted by a low frequency stimulus (Cond 3)? Their physical characteristics differ so widely And whereas the high frequency primaries generated a low frequency difference tone, their mere presence may have in some way lessened the effect on the response to the low frequency test stimulus There is no satisfactory solution to this dilemma, but we conducted additional tests, the outcome of which seemed to have attenuated the seriousness of the problem

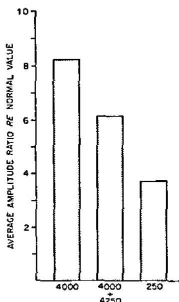


Fig 2 The relative amplitude of the response to the test stimulus as a function of the different intervening stimuli whose frequencies are expressed in Hz.

SUB-EXPERIMENT 1

Method

Again we investigated the influence of intervening stimuli on the response amplitude evoked by the test stimulus In Part 1, our intervening stimuli were 1) 4 000 plus 4 250 Hz presented simultaneously, and 2) 4 000 plus 250 Hz presented simultaneously The test stimulus remained at 250 Hz Intensity, duration, and rise fall time for the various frequencies were the same as those described for the main experiment The same listeners participated and each were given 2 test sessions A session was comprised of 2 presentations of each [illegible] condition in an ABBA [illegible] resents the 4 000–4 250 Hz [illegible] "B" represents the [illegible]

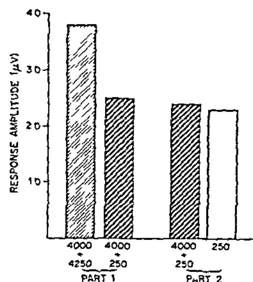


Fig 3 Response amplitude to the test stimulus under conditions of different intervening stimulation

pairing Responses evoked by 75 test stimuli were summed for each condition of intervening stimuli Part 2 was conducted identically except the intervening stimuli were 1) 4 000 plus 250 Hz presented simultaneously, and 2) 250 Hz presented alone

Results

regard to Part 1, the stimulus pair which pro- d the difference tone has a significantly less ect ($p < 0.01$) on the responsiveness to the 250 Hz test stimulus than did the stimulus pair, 4 000 plus 250 Hz, as indicated by the Wilcoxon Matched Pairs Signed Ranks test When apply- ing the Wilcoxon test to the Part 2 data, no differences were found between the 4 000 plus 250 Hz intervening stimulus pair and the inter vening 250 Hz tone with respect to suppressing the response to the 250 test stimulus Each had about the same effect A histogram based on mean response amplitudes to the test stimulus associated with those intervening stimuli used in Parts 1 and 2 is shown in Fig 3

Comment

To produce a maximal suppressive effect on the response to a 250 Hz test stimulus it apparently does not matter whether the 250 Hz intervening stimulus is paired with a high frequency tone, in this case, 4 000 Hz, or whether it is present alone The manner by which the 250 Hz in vening tone is generated, however, is critical must be contained in the auditory input for suppressive effect is significantly reduced if 250 Hz intervening "experience" is a cons ence of nonlinear distortion within the ear

SUB EXPERIMENT 2

Method

Another possible explanation for the finding t the intervening difference tone affected the sponse to the test stimulus less than did the Hz sinusoid is that the loudness of the diff ence tone may have been lower than that of 250 Hz tone Butler (1968) reported that an int vening stimulus which is softer than the t stimulus has a lesser suppressive effect than d an intervening stimulus whose loudness is eq to that of the test stimulus And it should pointed out that the method of best beats wh we used to set the intensity of the 250 Hz t stimulus as well as the 250 Hz intervening s mulus lacks precision There is a range of int sity values at which the beats appear equa loud or nearly so To assuage our doubts ran additional tests where the loudness of t difference tone was deliberately set above that the 250 Hz sinusoid Specifically, the 250 Hz te stimulus and the 250 Hz intervening stimul were reduced by 15 dB Since we obvious wanted to avoid distortion in our sound system we only boosted the 4 000 and 4 250 Hz pr maries by 2 dB Under these conditions, th difference tone was judged to be unquestionabl louder than the 250 Hz sinusoid With the sa 8 persons, we measured their response amplitud to the 250 Hz test stimulus when the 250 H sinusoid and when the 250 Hz difference ton served as the intervening stimuli Again 2 t sessions were given with each subject be tested on each experimental condition a total o four times

Results

Notwithstanding a clear difference in loud between intervening stimuli, the results were t

ame as those found in the main experiment All ... showed greater response amplitudes to he test stimulus when the stimuli producing the 50 Hz difference tone intervened between successive test stimuli than when the 250 Hz sinusoid intervened Mean response amplitude for former was 2 9 μV, that for the latter was 1 8 μV

DISCUSSION

Our main effect was unequivocal The stimuli producing the difference tone affected the response to the test stimulus less than did a sinusoid of the same frequency (250 Hz), but more than did one of the primaries (4 000 Hz) The magnitude of these differences exceeded the 1 percent level of confidence We infer then that the difference tone was processed differently than either 1) the primaries or 2) a sinusoid whose frequency was identical to that of the difference tone The data from additional tests rule out some possible explanations It was not the presence *per se* of a high frequency tone which attenuated the suppressive effect of the difference tone on the test stimulus If a 250 Hz sinusoid is present in the original stimulus, it will severely reduce the response amplitude to a 250 Hz test stimulus It matters not whether the stimulus complex also contains a high frequency tone (Sub-experiment 1) Nor was it that the difference tone may have been softer than the test stimulus, make it appreciably louder and it still does not affect the responsiveness to the test stimulus as much as a softer sinusoid identical in frequency (Sub-experiment 2)

Are there other data which suggest that a first-order difference tone is treated by the auditory periphery as neither one of the primaries which elicits the difference tone, nor as a sinusoid of the same frequency? There are, but they are generated by vastly different experimental procedures, viz, electro-physiological recordings of cochlear microphonics (CM) in the guinea pig cochlea DeBoer & Six (1960) published the original findings Dallos and his associates (Sweetman & Dallos, 1969, Worthington & Dallos, 1971) confirmed these findings and went on to develop a theoretical structure for them Their account runs as follows At relatively low intensity levels of the primaries, the CM corresponding to the difference tone reaches its maximum amplitude somewhat apically to the place where the higher primary CM is maximal At relatively high intensity levels of the primaries, the difference tone CM is distributed along the basilar membrane similarly to that of a sinusoid identical in frequency A two-stage process of cochlear distortion is proposed Stage 1, operating at the lower intensity levels reflects an electromechanical (hair cell transducing process) distortion Stage 2, operating at the higher intensity levels, reflects a hydromechanical distortion The latter process initiates its own traveling wave which then behaves as any other traveling wave initiated by a sinusoid of corresponding frequency Our data placed in this Stage 1 – Stage 2 context, would stand at the transition point They reflect neither stage unequivocally and the reason for this may be that the intensity of our primaries (80 dB SPL) falls in the middle of the range explored by Worthington & Dallos (50 to 115 dB SPL)

Worthington & Dallos, taking cognizance of their finding that the first-order difference tone CM can have its maximum at a place along the basilar membrane different from that of a CM elicited by a sinusoid of corresponding frequency, asked the question Is this difference tone audible? If we are at all justified in relating our data to theirs, the answer is "yes" Our difference tone was clearly audible and it obviously was not being processed in the same way as a sinusoid of corresponding frequency

ZUSAMMENFASSUNG

Die Amplitude der N_1–P_2-Komponente des evozierten Potentials fur einen 250-Hz Ton gemessen zwischen dem Vertex und den beiden Ohrlappchen, wurde in drei experimentellen Situationen untersucht Der Testton wurde alle 5 Sekunden einmal dargeboten. Dazwischen wurden — einmal pro Sekunde — entweder 1) ein 4 000-Hz Ton 2) gleichzeitig ein 4 000-Hz Ton und ein 4 250-Hz Ton was zu einem horbaren 250-Hz Differenzton fuhrte oder 3) ein 250-Hz Ton interpoliert Wir wussten von fruheren Untersuchungen, dass der 250-Hz Ton die

Amplitude des evozierten Potentials für den 250-Hz-Testton stärker reduzieren wurde als der 4 000-Hz Ton Die Frage war, ob das Tonpaar das den 250-Hz Differenzton veranlasste, in seinem Effekt dem 250-Hz-Ton oder dem 4 000-Hz-Ton gleichen wurde Die Resultate zeigten, dass die Amplitude der N_1-P_2 Komponente nach dem 4 000/4 250-Hz-Tonpaar grösser war als nach dem 250-Hz-Ton, aber kleiner als nach dem 4 000-Hz-Ton Wir folgerten, dass der Differenzton anders verarbeitet wird als ein Sinuston derselben Frequenz, und auch anders als ein Sinuston mit hoher Frequenz, wie er bei der Produktion des Differenztons mitwirkte In der Diskussion nahmen wir Bezug auf elektrophysiologische Studien der Cochlea, die offensichtlich vergleichbare Resultate lieferten

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J L Gorny, M D
Dept of Otolaryngology
Cook County Hospital
Chicago, Ill 60612, USA

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CORTICAL EVOKED POTENTIALS RECORDED FROM THE GUINEA PIG WITHOUT AVERAGING

R A Walloch

From the Kresge Hearing Research Laboratory, Department of Otolaryngology, University of Oregon Medical School, Portland Ore, USA

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Abstract Potentials evoked by tonal pulses and recorded with a monopolar electrode on the pial surface over the auditory cortex of the guinea pig are presented These potentials are compared with averaged potentials recorded in previous studies with an electrode on the dura The potentials recorded by these two techniques have similar waveforms, peak latencies and thresholds They appear to be generated within the same region of the cerebral cortex As can be expected, the amplitude of the evoked potentials recorded from the pial surface is larger than that recorded from the dura. Consequently averaging is not needed to extract the evoked potential once the dura is removed The thresholds for the evoked cortical potential are similar to behavioral thresholds for the guinea pig at high frequencies however evoked potential thresholds are elevated over behavioral thresholds at low frequencies The removal of the dura and the direct recording of the evoked potential appears most appropriate for acute experiments The recording of an evoked potential with dura electrodes employing averaging procedures appears most appropriate for chronic studies.

Several types of bioelectric potentials have been recorded from the human scalp (Davis, 1965, Cody & Bickford 1965, Cody & Klass, 1968, Rapin & Bergman 1969) These potentials have been especially useful with infants the mentally retarded and malingerers Similar potentials have been recorded from experimental animals in an attempt to quickly assess their auditory acuity

In order to test the auditory ability of animals thresholds for the evoked cortical potential have been obtained for the cat (Hind & Schuknecht, 1954, Kimura et al, 1956, Hattori et al, 1971) as well as for the guinea pig In the guinea pig, Djalilian & Cody (1973) and Hattori (1970) have used averaging techniques to obtain evoked potentials from dura electrodes In one study (Kern et al, 1969) the vertex response was recorded from the guinea pig In a number of recent studies (Walloch et al, 1971*a*, *b*, 1973*a*, *b*, 1974*a*, *b*, Cowden & Walloch, 1973) I have removed the dura and placed the recording electrode directly on the pial surface In this manner, the evoked cortical potential could be recorded without averaging The present paper compares and contrasts the records obtained from electrodes on the pia without averaging with those obtained from electrodes on the dura with averaging The data contained in the present paper have not been reported except as a brief communication (Walloch, 1971*a*)

METHOD

Eleven guinea pigs of the Topeka stock weighing 400–750 g were used in this study Each guinea pig had an active Preyer reflex The guinea pig was deeply anesthetized with diallylbarbituric acid (60 mg/kg) and urethane (240 mg/kg) In other studies (Walloch et al, 1974*a*, *b*) sodium pentobarbital (45 mb/kg) was used with equal success Rarely, a supplemental dose was needed to maintain anesthesia A tracheotomy was per

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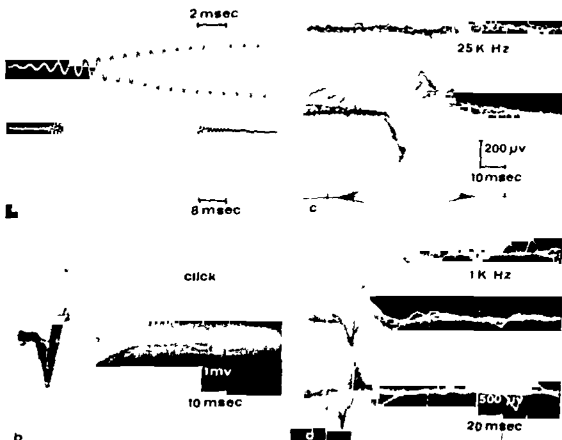


Fig 1 Record of the stimulus and evoked responses Negative is up (*a*) Averaged output of the calibrated microphone to a standard tonal pulse, showing that the sound pulse was free of significant on set transients (*b*) Superimposed cortical evoked responses to repeated ks (*c*) Cortical responses evoked by 25 kHz tonal Top tracing shows lack of evoked activity follow- repeated stimulus presentations at 21 dB SPL The middle tracing show a clear evoked response followi repeated stimulation at 31 dB The bottom line indica the duration of the tonal pulse (*d*) Cortical response 1 kHz tonal pulses The top line shows absence of corti activity following repeated stimulation at 25 dB T middle and bottom tracings show a well develop response at 35 and 45 dB respectively

formed and the animal was mechanically ventilated

In the present experiment the guinea pig's rectal temperature was continuously monitored (Yellow Springs Instrument 46 TUC) and maintained at 38 C When the body temperature falls below 35 C, the evoked cortical potential disappears The evoked potential returns upon warming the animal Above 40 C, the evoked potential deteriorates and in this case the deterioration is not reversible

Both pinnae were amputated to facilitate placement of the animal in a stereotaxic unit (David Kopf) The scalp and skull flap were removed to expose the entire left hemisphere

To remove the dura, an old tungsten micro- electrode was used, though any sharp thin prol would suffice The dura was impaled medial from the auditory area and lifted away from tl cortex An incision was made beneath the micr electrode with Vannas scissors This incision wa expanded anterolaterally The dura was r flected anteriad Some bleeding occurred o occasion while the dura was being remove When this occurred, it was best to procee quickly with the reflection of the dura With th dura reflected, the bleeding could then be con trolled with the use of gelfoam Most often th bleeding came from the soft tissue surroundir the cortical exposure Only rarely did the bleed ing stem from the cortex itself

After the dura was reflected, warm mineral o

was allowed to flow across the exposed cortex However the mineral oil was not found to be needed in subsequent studies A silver ball electrode (0 18 mm) was moved across the temporal area of the guinea pig in 1 mm steps The impedance of the electrode was about 2 kilohms At each cortical position studied the threshold for an auditory evoked potential was obtained at 18 frequencies ranging from 0 1 to 40 kHz

In order to obtain these evoked potentials sound was delivered through the hollow ear bar contralateral to the exposed cortex A General Radio wave analyzer (1900A) Monsanto digital counter (103A) Simpson voltmeter Grason Stadler electronic switch (892E) Grason Stadler interval timer (471 I) General Radio decade attenuator (1450) McIntosh power amplifier (M 210-B) and a specially designed power attenuator shaped the electrical signal which drove the Western Electric 555 speaker The speaker was connected to the hollow ear bar as a closed sound system The rate of stimulation was once every 2 seconds Each tonal pulse was 50 msec in duration and had a rise time of 10 msec At the end of each day s work a 1/4 inch Bruel & Kjaer calibrated microphone was substituted for the animal and the sound at the end of the hollow ear bar was thus measured (Fig 1*a*)

The evoked cortical potentials were initially amplified using a Keithley differential amplifier (103) The filters were set at 100 and 3 000 Hz The calibration of this amplifier has been previously described (Meikle & Copeland 1973) After the initial amplification the potentials were displayed on a Tektronix storage oscilloscope (564B)

RESULTS

Acoustically evoked potentials were recorded from a restricted area within the temporal region of the cerebral cortex Other investigators have identified this region as the auditory cortex (Ödkvist et al 1973 Ziegler 1964 Kayser & Legouix 1963) The first wave was always surface positive and it had a peak latency of 10–15 msec A surface negative wave with a peak

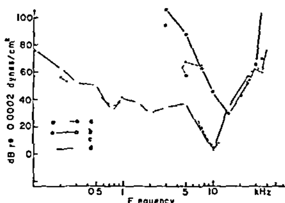


Fig 2 Threshold curves for the evoked responses recorded from four cortical positions in a single guinea pig All four positions were in the temporal region and within a few millimeters of each other See text for a fuller discussion of these data

latency of 20–40 msec generally followed the initial positive wave A second surface positive wave was observed rarely and only at very high sound intensities No later waves were observed

In Fig 1*b* an evoked potential to a click is shown Clicks were used in a few pilot animals to determine the location of the auditory cortex The waveform of the click evoked potential is highly similar to that evoked by tonal pulses No attempt was made to obtain the threshold of these click evoked potentials however they were very useful in determining the latencies of the evoked potentials

In Fig 1*c* the cortical responses to a 25 kHz tone are shown The top tracing shows a lack of an evoked response to repeated stimulation at 21 dB re 0 0002 dynes/cm² The middle tracing shows a 650 μV (p–p) response to repeated stimulation at 31 dB

In Fig 1*d* the cortical responses to a 1 kHz tone are shown In the top tracing no evoked activity is apparent following repeated stimulation at 23 dB In the middle trace a 1 mV potential was evoked by repeated stimulation at 33 dB These thresholds were stable over 5 and 6 hour recording sessions

In Fig 2 the thresholds for the evoked potential recorded from four positions on the auditory cortex of a single guinea pig are shown All

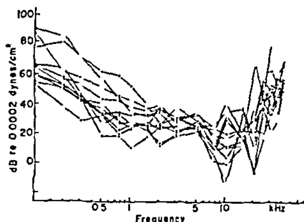


Fig 3 Thresholds for the evoked response from 11 guinea pigs The entire left hemisphere of each guinea pig was explored for evoked cortical activity At each frequency, the threshold represents the lowest intensity tonal pulse that evoked cortical activity regardless of the position of the recording electrode

four positions were within 4 mm of each other Two of the positions (*a* and *b*) were sensitive to high frequency tones, and tonal pulses below 1 5 kHz were totally ineffective in evoking a potential One position (*c*) was sensitive to low frequency stimuli One position (*d*) was sensitive to

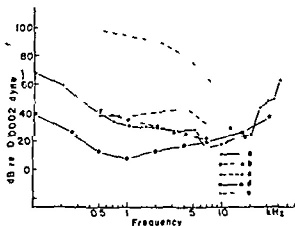


Fig 4 Comparison of the mean of the threshold curves plotted in Fig 3 with other measures of auditory acuity in the guinea pig (*a*) Mean of the threshold curves obtained in the present study (*b*) Thresholds for the averaged evoked potentials reported by Hattori (1970) from the dura overlying the auditory cortex of the guinea pig (*c*) Thresholds for the averaged evoked potentials recorded by *Djalilian (1973) from the dura overlying the* auditory cortex of the guinea pig (*d*) Behavioral threshold obtained by Miller (1966) in the guinea pig (*e*) Vertex potential threshold obtained by Kern (1969) from dura electrodes in the guinea pig

both low and high frequency stimuli C sequently, in order to obtain the lowest thr old for the evoked cortical potential a frequencies without averaging, a number of c cal positions must be sampled

In Fig 3, the thresholds for the auditory e ked potential obtained from 11 guinea pigs shown The entire left hemisphere was sam in 1 mm steps. At each frequency, the lo sound intensity that could evoke a cortical po tial regardless of the recording position is sho Consequently, each threshold curve repres potentials recorded from a number of cor positions

Fig 4 compares the mean of the thresh curves shown in Fig 3 with other comm measures of the auditory acuity in guinea p The thresholds obtained by computing avera evoked potentials recorded from the dura guinea pigs (Djalilian & Cody, 1973, Hatto Shoyama, 1970) are very similar to those tained in the present study These evoked po tial thresholds were similar to the behavi threshold of guinea pigs (Miller & Mur 1966) at high frequencies, but these evo potential thresholds were elevated over behav al thresholds at the low frequencies

COMMENT

The thresholds reported in the present study very similar to those reported by Hattori *Shoyama (1970) and to those reported* Djalilian & Cody (1973) (Fig 4) Hattori Djalilian used an averaging computer to rec evoke potentials from electrodes on the d over the auditory cortex The potentials corded in all three studies had, 1) similar wa forms, 2) latencies, 3) thresholds, and 4) spa distributions on the cerebral hemisphere C sequently the potentials recorded in the th studies appear to have the same origin

For certain acute experimental problems, t *removal of the dura and the direct recording* the evoked cortical potential appears m appropriate The large amplitude of the evok potential (Fig 1*c*, *b*) at threshold makes av

aging unnecessary Consequently, thresholds can be obtained much more rapidly with direct recording techniques than with averaging procedures While 50 stimulus presentations were used to obtain an averaged evoked potential in Djalilian's (1973) and Hattori's (1970) studies, only 2 stimulus presentations were needed in the present study

On the other hand, for certain chronic experimental problems, the recording of averaged evoked potentials from an electrode on the dura appears most appropriate The opening in the skull can be easily closed and the guinea pig may be allowed to recover from the anesthesia

The choice of the position on the cerebral cortex from which to record the evoked potentials greatly influences the thresholds obtained The position used by either Hattori (1970) or Djalilian (1973) appear appropriate for chronic studies For acute studies in which the dura has been removed, the cortex can be quickly searched for an appropriate position At each cortical position, the threshold for a potential evoked by 1 kHz and 10 kHz tonal pulses can be quickly ascertained If the 1 kHz threshold is below 45 dB and the 10 kHz threshold is below 35 dB, the cortical position is suitable for the experiment From such a position a complete threshold curve would appear similar to Fig *2d*

The behavioral threshold for pure tone stimuli have been obtained several times for the guinea pig (Horton, 1933, Anderson & Wedenberg, 1965, Miller & Murray, 1966, Heffner et al, 1971) The thresholds depicted in Fig. 4 *d* are representative of other behavioral determinations The thresholds for the evoked potentials obtained in the present study are similar to the behavioral thresholds at the high frequencies The evoked potential thresholds are elevated over the behavioral thresholds at the low frequencies The majority of the positions on the auditory cortex are most sensitive to high frequency stimuli While 63 % of the positions had best frequencies over 5 kHz, only 37 % had best frequencies below 3 kHz (Walloch, 1971 *c*) Perhaps this can explain the relative insensitivity of the evoked potential to low frequency stimuli

On the other hand, differences in 1) sound measurement procedures, 2) the rise time, and 3) duration of the tones cannot be discounted as contributing to the differences in the evoked potential and the behavioral thresholds

The vertex response of the guinea pig (Kern et al, 1969) is highly elevated over the other measures of auditory acuity shown in Fig 4 This contrasts with the human vertex potential which is similar to the thresholds obtained by conventional pure tone audiometry (Cody & Bickford, 1965, Cody & Klass, 1968, Rapin & Bergman, 1969) The reason for this difference between the guinea pig and human vertex potential is not apparent

The evoked potential from the auditory cortex has been used to study the effects upon the auditory system of intense sound stimulation (Hattori, 1970, 1971) ethacrynic acid (Walloch, 1974*a*) and mechanical lesions of the cochlea (Kimura et al, 1956, Hind & Schuknecht, 1954) The evoked potential has also been used to investigate various placements of electrodes for the electrical stimulation of the ear (Walloch et al, 1973*a*, 1974*b*) In the future, we can expect that the evoked potential will be used to study an ever-increasing scope of problems

ZUSAMMENFASSUNG

Aktionspotentiale der Horrinde wurden bei Meerschweinchen durch Tonimpulse hervorgerufen und mittels monopolarer Elektrode an der pia mater abgeleitet Sie wurden mit Durchschnittsaktionspotentialen verglichen die in fruheren Untersuchungen von der dura mater abgeleitet worden waren Diese mit verschiedener Methodik erhaltenen Potentiale ahneln einander hinsichtlich Verlaufsform maximaler Latenzzeit und Schwellenwert Sie scheinen in derselben Gegend der Hirnrinde zu entstehen Wie zu erwarten ist die Amplitude der von der pia mater abgeleiteten Aktionspotentiale grösser als die von der dura mater Daher ist es nicht notwendig Aktionspotentiale zu ermitteln wenn einmal die dura mater beseitigt ist Hervorgerufene Rindenpotentiale haben beim Meerschweinchen bei hoher Reizfrequenz ähnliche Schwellenwerte wie Verhaltungsreaktionen Jedoch liegen bei niedrigen Frequenzen die Schwellenwerte von hervorgerufenen Potentialen hoher als die Schwellenwerte von Verhaltungsreaktionen Beseitigung der dura mater und direktes Registrieren des Aktionspotentials scheinen am besten fur akute Experimente geeignet zu sein wahrend

Registrieren von Durchschnittsaktionspotentialen mit dura Elektroden sich am besten für chronische Experimente zu eignen scheint

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R. A. Walloch, Ph.D.
Dept. of Otolaryngology
University of Oregon Medical School
Portland, Ore. 97201, USA

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BIASING OF THE SUMMATING POTENTIALS

J D Durrant[1] and D Gans[2]

From the Auditory Research Laboratory, Northwestern University, Evanston, Ill, USA

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Abstract Summating potentials (SP) were recorded in the guinea pig cochlea, utilizing the differential electrode technique The effects of biasing the cochlear partition on the SP were observed under three different conditions 1 electrical biasing—direct current polarization 2. stimulus biasing—mechanical bias induced by low frequency sound stimulation 3 COCB biasing—electrical stimulation of the crossed olivo-cochlear bundle All three forms of biasing were found to have similar effects on the SP which can be described in terms of the 'absolute direction of change' in the potential Under negative current (scala vestibuli negative, scala tympani positive), the negative phase of the stimulus bias (deflection of the cochlear partition towards scala vestibuli) or stimulation of the COCB, the SP is made more positive or less negative, as the case may be In other words, the negative component of the differentially recorded SP is depressed whereas the positive component is enhanced The dominant influence of all three forms of biasing appears to be on mechanoelectric distortion arising in the hair-cell transduction process

The summating potential (SP) is by definition a distortion product It is typically observed as a d c response to a sinusoidal (a c) input, namely a pure tone sound stimulus (Dallos, 1973) Over the past several years a series of experiments have been carried out in this laboratory which were designed to analyse the relative contribution of mechanical and electrical nonlinearities to the generation of various components of the SP in the cochlea (Durrant & Dallos, 1972, 1974) The findings of these studies have at least contributed to the support of a unified concept of distortion production in the cochlea That is to say, the SP may be attributed largely to the same basic nonlinearity(ies) underlying such phenomena as harmonic distortion, intermodulation distortion, and interference observed electrophysiologically (Engebretson & Eldredge, 1968) It has also been suggested that the underlying distortion process(es) is strongly electromechanical in origin In other words, the distortion which is manifested in the electrophysiological responses of the cochlea appears to arise predominantly from a nonlinear (hair-cell) transducer process rather than a purely hydromechanical process such as nonlinear wave motion which leads to asymmetrical displacement of the basilar membrane and the formation of Bekesy's eddies (Tonndorf, 1970) That such hydromechanical nonlinearities may exist is not the point of argument, but rather it is asserted that components such as the SP arise at a later stage of cochlear function, namely at the site of the transducer

Electrical and mechanical forms of biasing of the cochlear partition can be observed to have similar effects on the SP, as suggested by the findings of two earlier studies—one by Butler & Honrubia (1963) and the other by Davis et al (1958) Data from these two studies are shown in Fig 1*a* and *b*, respectively The data have been replotted to show normalized response magnitude as a function of the independent variable The data presented in panel *a* demon-

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[1] Present address Temple University Health Sciences Center, 3400 N Broad St Philadelphia, Penn, USA

[2] Present address Speech and Hearing Clinic Kent State University, Kent, Ohio, USA.

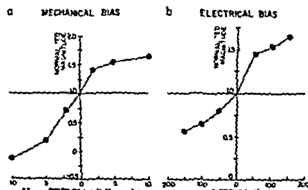


Fig 1 Effects of mechanical and electrical forms of biasing of the cochlear partition on the negative summating potential The graphs shown are adaptations of plots presented by (a) Butler and Honrubia (1963, composite of figures 1 and 2) and (b) Davis et al (1958 figure 7) (a) Normalized response magnitudes (see text) are plotted as a function of hydrostatic pressure selectivity applied to scala tympani (*ST*) and scala vestibuli (*SV*) (b) Normalized response magnitude as a function of current level Positive (+) current, SV positive and ST-negative Negative ( ) current, ST positive and SV-negative

strate the effects on the negative SP of selectively increasing the intracochlear hydrostatic pressure in either scala tympani or scala vestibuli The effects of applying direct current across the cochlear partition are shown in panel *b* (The normalized magnitude is obtained by simply viding the magnitude of the responses observed der the experimental condition by the magnitude under the control condition, that is under zero pressure or current) The SP may also be altered by electrically stimulating the crossed olivo-cochlear bundle Konishi & Slepian (1971*a*, *b*) have described these effects, but their data cannot be readily compared with those shown in Fig 1, since a direct analog of the "bias level" functions illustrated in this figure does not exist for the COCB biasing technique Current level alone does not determine the effectiveness of COCB stimulation which depends upon the use of pulsed current and the parameters of the pulses (level, duration, and frequency) Nevertheless, the fact that the SP can be changed under the influence of these different forms of biasing of the cochlear partition is impressive and suggests the basis of a useful comparative analysis This may lead to a more exacting means of determining the dominant form of nonlinea contributing to the production of the SP.

The studies discussed above leave sev questions open First, is the apparent comp ability of electrical and mechanical biasing illustrated by Fig 1, more than coincidence a secondly, is it equally applicable to both pos and negative components of the SP? Thi are the effects of COCB stimulation on SP in any way analogous to the effects of ele cal and mechanical biasing? In order to res these issues, it was necessary to examine effects of all three forms of biasing on the under a variety of stimulus and recording con tions The details of these investigations h been described elsewhere (Durrant & Dal 1972, 1974, Gans, 1974) The purpose of

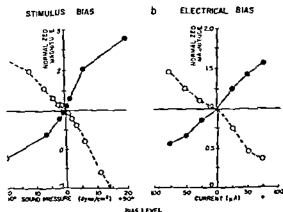
METHODS

Guinea pigs were utilized throughout th studies The animals were prepared with diff ential recording electrodes, generally in the f cochlear turn The tone burst elicited responses were averaged by means of a dig computer Since the probe stimuli were phase coherent tone bursts, the microphon elicited by the probe were cancelled thro averaging The sound stimuli were presen and monitored directly in a closed acous system

Three experimental techniques were utiliz to alter the SP, the details of which have be described elsewhere (Durrant & Dallos, 197 1974, Gans, 1974) Only a brief description provided here

1 D C polarization

Fluid filled pipettes were placed in the f cochlear turn of the guinea pig, one in sc vestibuli and the other in scala tympani near t site of recording Current was passed thro these electrodes (typically 50 μA) utilizi



g 2 Effects of different levels of bias on the positive) and negative (●) components of the DIF SP (*a*) IF⁻ was elicited by a 10 kHz tone burst presented at) dB SPL, DIF⁺, 1 kHz at 90 dB The bias stimulus as 70 Hz in the case of DIF⁻ and 35 Hz for DIF⁺ ıe abscissa shows the RMS sound pressure of the l ı mulus (*b*) DIF⁻ was elicited by a 10 kHz tone burst ıd DIF⁺ by a 200 Hz stimulus, both presented at 100 3 SPL.

ınstant current source The SP responses were ıcited with a 20 msec tone burst which will be ferred to as the probe stimulus

Stimulus biasing

'nder this procedure only recording electrodes ere utilized The probe stimulus was a brief ıne pip which was presented in the presence of a eady tone of much lower frequency The equency of the probe stimulus was always more ıan 10 times that of the bias stimulus The ıne pip (probe) was usually 2 or 5 msec long ith a rise-decay time equal to about one half its uration The brief transient like SP evoked y this stimulus exhibited characteristics not nlike that elicited by longer tone bursts with ctangular envelopes The occurrance of the robe could be synchronized with a set phase ı the low frequency bias stimulus Since the asilar membrane vibrates in phase along most ᶠ its length at low frequencies (Bekesy, 1960), ıe microphonic evoked by the bias itself served a means of selecting the appropriate phase the biasing deflection Usually, the probe as aligned with either the plus or minus 90° ınts of the relative CM phase

3 *COCB stimulation*

Following surgical preparation to reveal the dorsal aspect of the brainstem, a pair of wire electrodes were placed in the floor of the fourth ventricle between the facial genua Pulse trains fed through a photo-electric isolation unit were utilized to stimulate the COCB The acoustic probe stimulus (tone burst with rectangular envelope as in the d c polarization studies) was presented 10 msec following the end of the shock or bias stimulus

RESULTS

The results of the electrical and stimulus biasing experiments may be described in terms of bias-level functions similar to those shown in Fig 1, as seen in Fig 2 Data are presented for both the DIF⁻ and DIF⁺ components Results from the stimulus biasing studies are shown in panel *a* in which case the DIF⁻ was elicited by a 10 kHz tone pip presented at 90 dB SPL (sound pressure level re 0 0002 dyne/cm²), and DIF⁺ by 1 kHz at 90 dB It can be seen that the magnitude of the DIF component is enhanced (i e normalized magnitude >1) under the −90° biasing condition, but it is depressed (normalized magnitude <1) under the 90° condition The opposite is true for the positive component A comparable set of data from the polarization studies is presented in panel *b* In this instance DIF⁻ was elicited by a 10 kHz tone burst and DIF⁺ by 200 Hz, both presented at 100 dB SPL When scala vestibuli is made more positive with respect to scala tympani (+current), DIF⁻ is enhanced, and DIF⁺ depressed The opposite is true of negative currents Thus, the effects of stimulus and electrical biasing upon the negative SP component are quite similar to those evidenced in Fig 1, whereas the reverse of these effects are seen in the case of the positive component

The apparent differential effects of biasing on the two components of the DIF SP can actually be shown to represent an underlying relationship between them This relationship was previously described as the same *absolute*

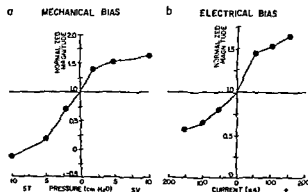


Fig 1 Effects of mechanical and electrical forms of biasing of the cochlear partition on the negative summating potential The graphs shown are adaptations of plots presented by (a) Butler and Honrubia (1963, composite of figures 1 and 2) and (b) Davis et al (1958, figure 7) (*a*) Normalized response magnitudes (see text) are plotted as a function of hydrostatic pressure selectivity applied to scala tympani (*ST*) and scala vestibuli (*SV*) (*b*) Normalized response magnitude as a function of current level Positive (+) current SV positive and ST negative Negative (−) current ST positive and SV negative

strate the effects on the negative SP of selectively increasing the intracochlear hydrostatic pressure in either scala tympani or scala vestibuli The effects of applying direct current across the cochlear partition are shown in panel *b* (The normalized magnitude is obtained by simply dividing the magnitude of the responses observed under the experimental condition by the magnitude under the control condition, that is under zero pressure or current) The SP may also be altered by electrically stimulating the crossed olivo-cochlear bundle Konishi & Slepian (1971*a*, *b*) have described these effects, but their data cannot be readily compared with those shown in Fig 1, since a direct analog of the "bias level" functions illustrated in this figure does not exist for the COCB biasing technique Current level alone does not determine the effectiveness of COCB stimulation which depends upon the use of pulsed current and the parameters of the pulses (level, duration, and frequency) Nevertheless, the fact that the SP can be changed under the influence of these different forms of biasing of the cochlear partition is impressive and suggests the basis of a useful comparative analysis This may lead to a more exacting means of determining the dominant form of nonlinea contributing to the production of the SP

The studies discussed above leave sev questions open First, is the apparent comp ability of electrical and mechanical biasing illustrated by Fig 1, more than coincidence a secondly, is it equally applicable to both posi and negative components of the SP? Thir are the effects of COCB stimulation on SP in any way analogous to the effects of elec cal and mechanical biasing? In order to reso these issues, it was necessary to examine effects of all three forms of biasing on the under a variety of stimulus and recording co tions The details of these investigations h been described elsewhere (Durrant & Dal 1972, 1974, Gans, 1974) The purpose of present writing is to present a direct compari of the effects of the three forms of biasing on SP recorded with differential electrodes

METHODS

Guinea pigs were utilized throughout th studies The animals were prepared with dif ential recording electrodes, generally in the f cochlear turn The tone burst elicited responses were averaged by means of a dig computer Since the probe stimuli were phase coherent tone bursts, the microphon elicited by the probe were cancelled thro averaging The sound stimuli were presen and monitored directly in a closed acous system

Three experimental techniques were utili to alter the SP, the details of which have be described elsewhere (Durrant & Dallos, 19 1974, Gans, 1974) Only a brief description provided here

1 D C polarization

Fluid filled pipettes were placed in the fi cochlear turn of the guinea pig, one in sca vestibuli and the other in scala tympani, near t site of recording Current was passed throu these electrodes (typically 50 μA) utilizing

able I *Mechanical and electrical events in the cochlea associated with various parameters of biasing and the cochlear electrical potentials*

Parameter	Corresponding event	
I Mechanical bias		
A Intracochlear pressure	ST pressure increase	SV pressure increase
B Sound	Rarefaction	Compression
II Basilar membrane displacement	Toward SV	Toward ST
III CM output	Neg phase	Pos phase
Electrical bias	Neg Current	Pos Current
V COCB bias	Activated	—
VI EP	Decreased	Increased
VII SP		
A DIF	Decreased	Increased
B DIF+	Increased	Decreased
C Direction of change	More pos	More neg

n the endocochlear potential (EP) From the of the microphonic elicited by the low frequency biasing stimulus and the expected change in the EP due to the polarization current Honrubia & Ward, 1969) and COCB stimulation (Fex, 1967) it can be surmized that the effects of all three forms of biasing reflect lectrically similar and related events Table I des a comparison of the corresponding vents underlying all of the experimental procedures reported here, including the mechanical biasing technique of Butler & Honrubia (1963) and other pertinent electrophysiological events ncreased pressure applied to scala tympani nd the rarefaction phase of the biasing sound wave both lead to deflection of the basilar membrane toward scala vestibuli Coincidenally, the negative phase of the microphonic is mirrored s an effective decrease in the EP Both negative current polarization and COCB stimulation bring about a reduction in the EP The net effects are such that when a particular biasing ondition is associated with a decrease in the EP, the DIF SP responses are made more positive This is true whether the change in the EP is brought about directly by an externally pplied current or indirectly by deformation of the cochlear partition or hyperpolarizing membrane current flow at the base of the hair cell which presumably results from efferent activity It follows that when the EP increases, the SP becomes more negative This can be brought about under electrical or mechanical forms of biasing but not under COCB stimulation

That there are considerable qualitative similarities between the effects of stimulus, electrical, and COCB biasing on the SP does not mean that they are perfectly analogous There are various differences between their overall effects, especially as seen in their individual influence upon the cochlear microphonic Electrical biasing simply causes an increase or decrease in the CM according to the direction of current flow—positive polarization (scala vestibuli positive) enhances, and negative (scala vestibuli negative) depresses the CM Under stimulus biasing the net effect is typically one of depression of the CM under most conditions or, in other words, an interference effect COCB stimulation causes the CM to be "paradoxically" increased (Fex, 1967 Sohmer, 1965), and these effects on the CM are limited to certain frequencies (Konishi & Slepian, 1971*a*) However, even in the face of such diversity underlying similarities prevail Under all forms of biasing the changes induced in the CM, if any, are much less dramatic than those observed in the SP

It might be suggested that the three forms of bias incorporated in these studies act at three different levels of the hair cell transduction system Invoking a slightly modified Davis model (1965), the CM generator may be invisioned as a nonlinear modulated resistance The following modes of action due to the different biasing procedures may then be conceived (1) Electrical biasing acts to alter the resting current driven through the modulated resistance (2) Stimulus biasing serves to alter the quasi steady state value of the resistance (3) COCB stimulation, by altering the membrane polarization, influences the load on the resistance Although the net effect of the biasing would be of the same nature, that is the manipulation of the electrical operating characteris-

tics, the detailed effects would not necessarily be identical due to the different ways in which the bias and the probe might interact under the different biasing paradigms

At this time one can only speculate about such matters, but the results of the studies reported here do offer compelling evidence that there is at least a strong underlying relationship between the effects of electrical polarization, stimulus biasing, and COCB stimulation By virtue of the presumed selective electrical effects of d c biasing and COCB stimulation, it may be concluded that the SP appear to arise from electromechanical nonlinearities In other words, SP production is dominated by distortion in the hair cell output Although the underlying mechanisms are still not clearly understood, it would seem that both the DIF+ and DIF− SP components are attributable to this same basic distortion process

ZUSAMMENFASSUNG

Unter Anwendung der Differentialelektrodentechnik wurde das summierende Potential (SP) der Meerschweinchenschnecke aufgezeichnet Die Auswirkungen der Beeinflussung von der Schneckenteilung auf das SP wurden unter drei verschiedenen Bedingungen beob- [illegible] 1 Elektrische Beeinflussung — direkte Strom[illegible]risation, 2 Erregungsbeeinflussung — mechanische [illegible]ebung, veranlasst durch Niederfrequenz Laut[illegible]ung, 3 COCB Beeinflussung — elektrische Reizung des gekreuzten „olivo-cochlear"-Bundels Es zeigte sich, dass alle drei Arten der Beeinflussung ahnliche Auswirkungen auf das SP hatten die als die „absolute Richtungsänderung im Potential bezeichnet werden können Bei negativer Stromrichtung (scala vestibuli negativ, scala tympani positiv), der negativen Phase der Erregungsbeeinflussung (Ablenkung der Schneckenteilung zur scala vestibuli) oder bei der Reizung des COCB wird das SP jeweils entweder stärker positiv oder schwächer negativ Mit anderen Worten, die negative Kom- [illegible]

scheint auf der mechano-elektrischen Verzerrung zu beruhen, die vom Haarzellenübertragungsprozess herrührt

ACKNOWLEDGEMENT

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J D Durrant, Ph D
Temple University Health Sciences Center
3400 N Broad St,
Philadelphia, Penn 19140, USA

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MACROMOLECULAR TRANSPORT BY THE MIDDLE EAR AND ITS LYMPHATIC SYSTEM

D J Lim and B Hussl[1]

From the Otological Research Laboratories, Department of Otolaryngology, Ohio State University College of Medicine, Columbus, Ohio, USA

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stract The macromolecular transport in the middle mucosa of the guinea pig was investigated by means light and electron microscopy using tracer substances h as Evans blue, India ink, and horseradish peroxidase RP) HRP particles were readily taken up by the middle mucosa and Eustachian tube They were absorbed all cell types, including ciliated, secretory and non retory cells The particles were first taken up by the ocytotic vesicles and then transported into the inter lular spaces by reverse pinocytosis These particles re transported toward the connective tissue through

RP in these lymph nodes were mainly found in the ecific granules of the macrophages

he normal middle ear mucosa is known to sorb soluble and particulate matter (Bortck & Proud, 1965, Breuninger & Feine, 1968, strahe & Westphal, 1971, Haye, 1972) However, only sketchy information is available about e lymphatic drainage from the middle ear in imans (Rouviere, 1932, Graves & Edwards, 44) and even less in laboratory animals

Absorption by the mucous membrane has an portant bearing on the development of musal and general immunity It is well established that most antigenic substances that come into contact with the mucosal lining of the respiratory and gastrointestinal tract are macromolecules such as proteins and mucopolysaccharides (Waldman, 1970) The present experiment was undertaken to elucidate the mechanism(s) involved in the uptake and processing of various macromolecules by the mucosa of the middle ear and Eustachian tube, and the regional lymph nodes

MATERIALS AND METHODS

Healthy guinea pigs weighing between [illegible] 700 gm were used in this experiment The [illegible] mental procedures were performed [illegible] tion anesthesia with methoxyflurane [illegible] fane®)

Three tracer substances [illegible] absorption studies of the middle ear

(1) *Evans blue* The [illegible] pigs were instilled through [illegible] branes with 0 2 ml of [illegible] Chilcott, Morris Plains, [illegible] auditory canal of the [illegible] was filled with dye to [illegible] external canal caused sta[illegible] nodes The animals were [illegible] 96 hours after dye in[illegible]on, the dye intracardially with [illegible] and traces glutaraldehyde The [illegible]ubes and in moved and pieces of [illegible]rifice In the auditory tubes were d[illegible] ear mucosa

[illegible] sicles [illegible] al cell [illegible] e tissue [illegible] rovilli

pported in part by the research grants from NIH NDS NS-08854-04 and the Deafness Research Founda n

[1] On leave of absence from Department of ENT University of Innsbruck, Austria.

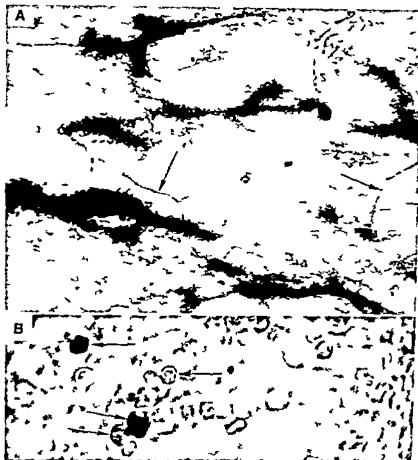


Fig 1 (A) A phase contrast micrograph shows the surface view of the gu nea p g m ddle ear mucosa 30 mın after tıllation of Evans blue into the bulla Dark channel e streaks are formed by heavy accumulat on of the Small blood cap llaries are ind cated by arrows 60 (B) A high magn f cat on phase contrast micro-graph shows numerous t ssue macrophages (hist o in the connective tissue layer of the gu nea p g m ear mucosa Several histiocytes, indicated by arrows up dye 60 m n after inst llat on of Evans blue int bulla. ×250

microscope (Zeiss) The lymph nodes in the neck which drain the middle ear and auditory tube were identified by bluish stains These nodes were removed and routinely processed for histological examination Whole pieces of the tym panic mucosae were dissected and mounted for surface examination

(2) *India ink* In 3 guinea pigs 0 2 ml of di luted black India ink (1 3 with sterile normal saline) was injected bilaterally into the tympan ic cavities The animals were kept alive for 24 48 and 62 hours The temporal bones and lymph nodes were dissected as described above The tissue was fixed in formalin and routinely processed for histological evaluation

(3) *Horseradish peroxidase (HRP)* 0 15 ml of HRP solution (30 mg HRP in 0 5 ml no saline) (Type II Sigma Chemical Comp St Louis molecular weight 33 000) was inj ed into the left tympanic cavities of 14 gu pigs The animals were sacrificed after 5 15 30 and 60 min following HRP instillation were perfused intracardially with 2% glu aldehyde Pieces of the tympanic mucosa the Eustachian tube were dissected in all mals In 4 guinea pigs the retroauricular junctional lymph were nodes also removed T specimens were processed according to the K novsky technique as modified by Duvall Sutherland The tympanic mucosa and tubes and lymph nodes from the right sides the animals served as controls



g 2
r mu
e mu ... ue ol
he epithelium Fifteen minutes after the introduction of HRP, the particles wer (P) of the ciliated ce (B), intercellular spa layer (CT) BM ba... odies MV, micro- HRP particles were esicles (PV) 30 min RP particles did not mplex (JC) between ell (C)

RESULT

Middle ear

Evans blue and India ink were both noted in the middle ears up to 96 hours after instillation into the bulla A was accumulated of dye were se the nasophar whole mount



Fig. 3 (A) Thirty minutes after instillation into the bulla, the HRP particles were seen in the lysosomal bodies (*Lys.*) of epithelial cells (*Epi*) and in the lymphatics (*LC*). *BM*, basement membrane; *CT*, connective tissue. (B) Sixty minutes after the bullar instillation, the HRP particles were seen in lysosomal bodies (*Lys*) of squamous type *mucosal epithelium (Epi). Some diffuse HRP particles* were noted in the connective tissue layer (*CT*), bu a few particles were found on the surface of the epi cells (C) Thirty minutes after the bullar instillatio HRP particles were found in the apex and peripher secretory (*S*) cell and in the multivesicular body (M a ciliated cell (*C*) MV, microvilli

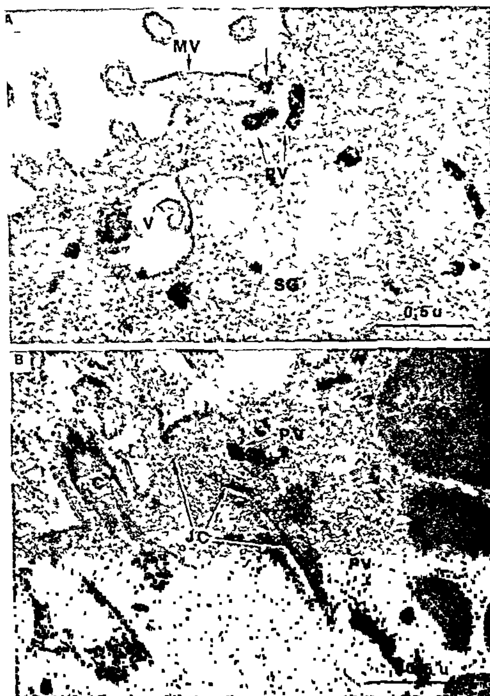


Fig 4 (A) A portion of the free surface of a secretory cell shows numerous pinocytotic vesicles (*PV*) which contain HRP particles 30 min after instillation with HRP suspension into the middle ear The HRP particles appear to adhere to the cell surface first and then a pit containing adhered HRP particles is formed at the base of the microvilli (*arrow*) Some pinocytotic vesicles fuse with a large vacuole (*V*) which contains dark bodies *MV*, microvilli *SG* secretory granule (B) The HRP particles were mainly taken up by the pinocytotic vesicles (*PV*) 30 min after instillation of the bulla The HRP particles did not pass through the intact junctional complex (*JC*) between a secretory cell (*SG*) and a ciliated cell (*C*)

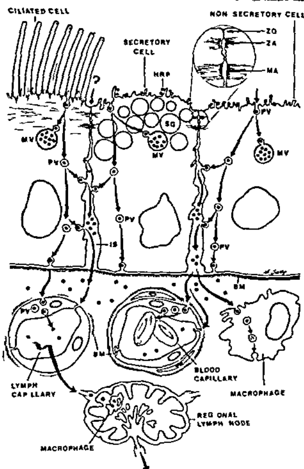


Fig 5 A schematic diagram illustrates the mechanisms of cellular transport of macromolecules (*HRP*) by the middle ear mucosa The major system for uptake of the [mo]lecules by the epithelium is accomplished by [pinoc]ytosis (*PV*) of the cellular membrane facing the [middle] ear cavity Then they are either stored in the [mu]ltivesicular body (*MV*) or transported into the inter[c]ellular space (*IC*) or transported across the cell toward the basement membrane (*BM*) The HRP does not pass through the junctional complex which includes zonula occludens (*ZO*) zonula adherens (*ZA*) or macula adherens (*MA*) However the HRP readily passes through the basement membrane of the epithelial cells The tissue macrophages take up large quantities of HRP in the connective tissue layer Some of the HRP enters into either the lymphatics or blood capillaries by pinocytosis and through the tight junctions Once the particles enter the lymphatics they are transported into the regional lymph nodes where the lymph macrophages take up most of the transported HRP

of 2 animals injected with Evans blue showed irregularly arranged blue streaks resembling channels that were independent from the capillary system (Fig 1*A*) Whether or not these channels represent lymphatic capillaries could not be determined India ink particles were found ly in the mucous blanket in the middle ear Eustachian tube, some were free and some taken up by macrophages present in the epithelial connective tissue of the middle and Eustachian tube (Fig 1*B*) Only a scattered India ink particles were detected in lymphatics and blood capillaries

The tracer particles were found in the ep lial cells and in the subepithelial conne tissue within 5 to 15 min following the instill of the bulla with HRP (Fig 2) In the specimens, the connective tissue layer was s ated with HRP particles, some of them also seen in lymphatics (Fig 3*A*) The 60 specimens showed very little HRP particl be present on the surface of epithelial cells majority of them were found in the conne tissue and in the lysosomal bodies of the ep lial cells (Fig 3*B*)

Uptake of HRP occurred in all epithelial types including secretory, non secretory ciliated cells (Fig. 3*B* and 3*C*) The mod HRP uptake by epithelial cells was accompli by pinocytosis First, particles adhered to surface of microvilli, then the cytoplasm in nated to form a vesicle containing these ticles (Fig 4*A*) The particles did not through the intact tight junctions of the epi lium (Fig 4*B*) The particles transported by vesicles appeared in (1) intercellular spaces lysosomes and multivesicular bodies, and (3) connective tissue layer, as illustrated in a s matic drawing (Fig 5) The particles that tered into lysosomes or multivesicular bo appeared to remain in the cell within the exp mental period There was no evidence, howe that the particles can be trapped at the leve the epithelial basement membrane, previo considered as a barrier for HRP as noted Haye (1972) The HRP particles that had fused into the connective tissue appeared to ac mulate near the collagen fibers but not r reticular fibers (Fig 6) Some HRP particle the connective tissue were also taken up histiocytes But large amounts of tracer parti were transported into lymphatics and bl



Fig 6 A close up view of the basal portion of the mucosal epithelium shows intense HRP reaction in the intercellular spaces (*ICS*) in dark bodies (*DB*) and in the connective tissue This HRP was observed 30 min after middle ear instillation with HRP suspension Heavy accumulation of HRP can be noted near the collagen fibers (*CF*) but the reticular fiber area (*RF*) is free of the particles. M mitochondrion

capillaries The passage of these particles across endothelial cells was accomplished mainly by pinocytosis (Fig 7*A*), however, some HRP passed through the intact endothelial cell junction

Special attention was paid to whether squamous epithelial cells of the mucosa transported more HRP than columnar epithelial cells However in the present investigation, the differences in the HRP uptake by these cells were inconsistent and could, therefore, not be evaluated with certainty

Some of the epithelial cells that seemed to be in a stage of degeneration, picked up large quantities of HRP in their cytoplasm These cells were either ciliated or secretory and often appeared to be compressed by surrounding ones The HRP

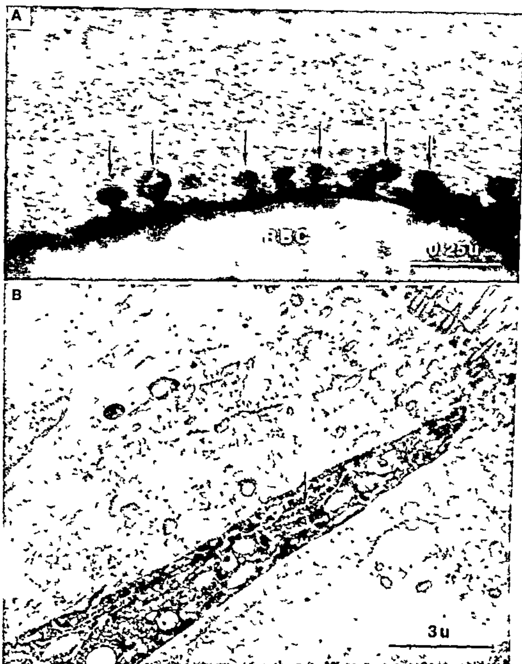


Fig 7 (A) An electron micrograph of the capillary wall of a blood vessel in the middle ear mucosa shows numerous pinocytotic vesicles (*arrows*) These vesicles contain HRP in the endothelial cell facing the lumen one hour after middle ear instillation with HRP suspension A portion of a red blood cell (*RBC*) is shown (B) accumulation of HRP in an epithelial cell can b 30 min after the HRP instillation On a morpho basis this cell appears to be a degenerating secreto

reaction products in these cells were distributed diffusely in the cytoplasm and not confined to the cytoplasmic vesicles (Fig 7*B*)

No cytotoxic effect caused by HRP was noticed in the present experiment, and the peroxidase reaction was not observed in control mens incubated without substrate except the peripheral part of the red blood cells in capillaries

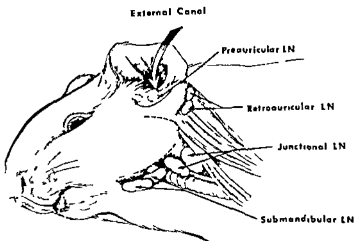


Fig 8 A sketch of the upper cervical lymph nodes of the guinea pig LN, lymph node Drawing by Nancy Sally

Regional lymph nodes

or gross observation, Evans blue yielded the est results for identifying the draining lymph odes by their pale blue coloration While the re auricular and submandibular lymph nodes ere not stained with Evans blue following iddle ear instillation, the retro auricular and unctional lymph nodes (LN) were positively lentified as those draining the middle ear and ie tube In two cases, the afferent lymphatics ading into the retro auricular lymph nodes ere clearly outlined The term "junctional odes' suggested by Fisch & Sigel (1964) to enote a group of lymph nodes situated below nd behind the mandibular angle, as illustrated i Fig 8, was used in the present investigation vans blue was not picked up by regional ymph nodes, when only the external ear canal as instilled with dye

For histological examination, the India ink nd HRP yielded the best results Evans blue ould not be detected on unstained sections of nose lymph nodes that had macroscopically iken up the dye

The tracer particles were detected in the ipsi iteral retroauricular and junctional lymph nodes s early as 5 min following the tympanic in- illation with HRP More particles were present i the former than in the latter In the 5 min roup, the particles were mainly present in the ibcapsular sinuses, but also in capillaries and i the connective tissue of the cortex, mostly taken up by macrophages (Fig 9*A*) After longer survival times, macrophages in the medullary part of the lymph nodes had also ingested HRP particles (Fig 9*B*) The number of HRP ingest- ed macrophages was proportionally increased as the length of the survival time of the animal extended At the ultra structural level, reaction products were found to be mainly confined to the specific granules of macrophages (Fig 10)

DISCUSSION

The present investigation confirmed earlier re ports that the middle ear mucosa performs an absorptive function (Bortnick & Proud, 1965, Breuninger & Feine, 1968, Terrahe & Westphal, 1971, Haye, 1972) This function depended large- ly on the size of the foreign particles, since only a few carbon particles of India ink, with an average diameter of 400 Å, were taken up, where- as, HRP particles (diameter 50–60 Å) readily passed through the epithelium Therefore, the mode of absorption and transcellular transport of macromolecules was mainly studied using HRP as a tracer

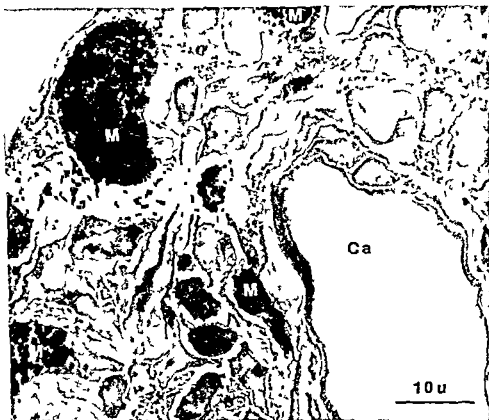
It is now established that the epithelium of normal middle ear mucosa of human and ani- mal alike consists of ciliated, secretory and non- secretory cells and that the latter, in general, are squamous cells possessing varying numbers of microvilli on their surfaces (Sade, 1966, Lim et al, 1967, Kawabata & Paparella, 1969, Hussl



Fig 9 (A) A phase contrast micrograph of a junctional lymph node 15 min after HRP instillation of the bulla The black deposits (HRP reaction) are mainly in the *macrophages of the subcapsular area and in the macrophages of the cortex* Some HRP particles can be seen in the sinus ?50 (B) Thirty minutes follow instillation of the bulla the particles were abu many of the macrophages located in the medul *junctional lymph nodes ?50*

& Lim 1969 *Hentzer 1970* Kaneko et al 1971 Hilding & Heywood 1971 Lim et al 1972 Shimada & Lim 1972)

The present experimental evidence showed that absorption and transcellular transport of HRP was accomplished by all epithelial cell types The HRP particles were taken up by epithelial cells with pinocytosis and then mainly *transported to the intercellular space by* pinocytosis although some of them wer up by lysosomes and multivesicular bodi absorptive mechanism is similar to that body transport across the proximal abs cells in the small intestine of the neon (Rodewald 1973) and that of ferritin tr across Reissner s membrane (Hinojosa



g 10 Among numerous lymphocytes several macrophages (*M*) containing HRP can be seen in the junctional lymph nodes of the guinea pig 15 min after bullar instilla t on with HRP suspens on *Ca* cap llary

Ve could not confirm the earlier report that the acer particles passed through the tight junc ons of the epithelial cells (Terrahe & Westphal 971 Haye 1972) The HRP particles freely assed through the intact epithelial basement embrane which did not constitute a barrier or the diffusion of HRP as noted by Haye (1972)

The collagenophilic and reticulophobic prop-rties of the tracer particles (HRP) observed the present experiment could not be explained However due to this peculiarity patchy accu ulation of the tracer particles was noted in he connective tissue layer

The mechanism involved in transport of HRP icles across the endothelial cells of the lymph nd blood capillaries in the middle ear was milar to that in the capillaries of stria vascularis Duvall & Sutherland 1972) where the tracer articles were mainly transported by pinocytosis nd reverse pinocytosis But also some particles passed through the tight junction of the endo thelial cells as observed by Karnovsky (1967) in the vessels of skeletal and cardiac muscles

Although the precise route of the draining lymphatics from the tympanic cavity and the tube were not clearly demonstrated in this study the present finding strongly suggests that retroauricular and junctional lymph nodes are the major lymph drainage system for these areas in the guinea pig This finding is in agreement with the report by Arnold et al (1972) They observed small particles injected into the sub-arachnoid space were found in the submucosal connective tissue of the middle ear and drained by the regional lymph node (retro-auricular node) Their finding confirmed an earlier experi ment conducted by Karbowski (1930) also using guinea pigs The regional lymph nodes in our study were stained as early as 5 min after in stillation of Evans blue into the bulla. In the

human, the lymphatics of the middle ear and Eustachian tube are thought to drain to the retropharyngeal, pharyngeal, parotid, submandibular and upper deep cervical lymph nodes (Rouviere, 1932, Graves & Edwards, 1944) Preauricular, parotid, submandibular and jugular lymph nodes were not stained with Evans blue in the present experiment The retropharyngeal lymph node described in the human could not be identified in the guinea pig

It is known that histiocytes (tissue macrophages) are present in normal middle ear mucosa, in infected ears (Lim & Klainer, 1971) and in ears with serous otitis media (Lim & Birck, 1971, Lim et al, 1972) While it is apparent that histiocytes engulf a large amount of HRP particles in the middle ear, there is no evidence that the number of histiocytes increased as the result of HRP instillation However, in the regional lymph nodes, the number of macrophages appeared to increase in direct proportion to the length of time between the HRP instillation and the sacrifice of the animal In 5-min specimens, the HRP particles were mainly present in macrophages in the subcapsular sinuses But in the 30- and 60 min groups, an increasing number of macrophages in the medullary area ad taken up the tracer particles This finding ggests that as the lymph reaches the subcapsular sinuses through the afferent lymphatics, part of the transported particles are ingested by macrophages, and as the lymph passes through the node from the cortical to the medullary areas, more particles are phagocytized by macrophages We can speculate that the excess tracer particles will then be carried from the lymph node via the efferent lymphatics to the next lymph nodes

Mucosal immunity is based upon the presence of a local immunological defense system in the mucous membranes of the respiratory and gastro-intestinal tract This concept had been postulated by Besredka in 1927, but was substantiated only in the last decade (Waldman, 1970, Tomasi, 1972) Recent evidence has shown that the middle ear mucosa is also endowed with such a defense system (Bernstein et al, 1972, Ishikawa et al, 1972, Lim et al, 1972, Mog al, 1973) A prerequisite for the function this system is that foreign substances (mac molecules) can be absorbed by the lining muc and then processed by macrophages Th processed substances can act as antigens to stir late antibody-forming cells (Pearsall & Wei 1970)

The present experiment and the results other investigators (Terrahe & Westphal, 19 Haye, 1972) confirm that the middle ear m cosa can take up large quantities of mac molecules and can process them by macropha present in the mucosal level and also in t regional lymph nodes This finding further ports the notion that the middle ear is provi with an immunological defense system

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ZUSAMMENFASSUNG

Unter Anwendung verschiedener Tracer Substa (Evans Blau Tusche Meerrettich Peroxidase) die Meerschweinchen durch das Trommelfell in Pauke Bulla instilliert wurden, konnte die Resorptionsleist der gesunden Mittelohrschleimhaut und der Abtransp der Markierungssubstanzen in die regionären Lym knoten auf licht und elektronen mikroskopischer E untersucht werden Wahrend Tusche und Peroxid losung sich als geeignete Markierungssubstanzen fur histologische bzw ultrastrukturelle Untersuchung wiesen, diente Evans Blau der makroskopischen Ide fizierung der abhängigen Lymphknoten

Die resorbtive Leistung der Mittelohrschleimh hängt von der Grosse der zu resorbierenden Partikel Wahrend Tusche Teilchen (Durchmesser etwa 400 nur in geringem Masse in das Epithel aufgenommen Grossteil aber durch die Tube abtransportiert wurd wurden Peroxidase Partikel (Durchmesser 50–60 rasch von der Schleimhaut resorbiert Das Tracer Prot wurde von allen Epithelzell Typen durch Pinozytose genommen und vorwiegend in die Interzellular weitertransportiert Während die epithelialen Schl leisten eine Barriere fur das Eindringen des Tra Proteins darstellen, kann dieses die Basalmembran Epithels ohne Schwierigkeiten passieren Im subepith len Bindegewebe wurde das Reaktionsprodukt vorwieg um kollagene Fasern in Histiozyten und in gro Menge in Blut und Lymphkapillaren festgestellt

Bereits 5 Minuten nach Instillation von Peroxidase-

pillaren und Makrophagen des Kortexbereiches fand, zeigte sie sich nach längeren Überlebenszeiten nach 30 Minuten und mehr vor allem in Makrophagen in den zentralen Abschnitten der Lymphknoten

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D J Lim, M.D
Dept of Otolaryngology
Ohio State University Hospitals
456 Clinic Drive
Columbus, Ohio 43210, USA

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PROTEINKONZENTRATION DER MEERSCHWEINCHEN-PERILYMPHE NACH SCHALLBELASTUNG

F Scheibe, U Hache, H Haupt und H -J Gerhardt

Aus der Hals Nasen Ohren Klinik des Bereichs Medizin (Charité) der Humboldt Universitat zu Berlin Berlin DDR

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Abstrakt Die Proteinkonzentration der Perilymphe larmbelasteter Meerschweinchen wurde mit Hilfe einer fruher beschriebenen Mikromethode untersucht Die Tiere wurden einseitig in einem akustisch geschlossenen System 1 Stunde mit Breitbandrauschen der Intensität von 140 dB beschallt Die Perilymphe wurde zu verschiedenen Zeiten nach Beschallungsende entnommen Sofort nach 6 und 24 Stunden Bei den beschallten Tieren ist es wesentlich problematischer, blutfreie Perilymphe zu gewinnen als beim Normaltier Selbst post mortal ist es schwierig blutfreie, nicht hamolytische Proben zu gewinnen, besonders aus der Scala tympani *Ein Teil der Tiere wurde deshalb vor dem Dekapitieren* intraarteriell mit Ringer Losung bzw Infukoll® M 40 perfundiert Nach der Beschallung ist nur bei den intra arteriell perfundierten Tieren eine geringe Erhohung der *Proteinkonzentration in der Perilymphe der beschallten* Ohren nachweisbar Der Konzentrationsunterschied ist bei den 6 Stunden nach Beschallungsende entnommenen Proben am deutlichsten Ein sicherer Hinweis auf die erkunft dieses Proteins konnte aus den Untersuchungen nicht gewonnen werden

Unsere fruheren Untersuchungen (Scheibe et al, 1972) haben gezeigt, *daß eine qualitative* Änderung des Proteinmusters der Perilymphe (PL) nach horschädigender Schallbelastung im*munelektrophoretisch nicht nachweisbar ist* Das schließt nicht aus, daß sich die Proteinkonzentration der PL durch die Beschallung ändert Bisher wurden dazu nur orientierende Untersuchungen an Kaninchen (Miyake, 1960) und Katzen (Komarovich & Plouzhnikov, 1966) mit unterschiedlichen Ergebnissen durchgeführt Wir haben deshalb unsere qualitativen Untersuchungen larmbelasteter Meerschweinchen quantitative Untersuchungen ergänzt Grundlage dienten vorangegangene Unte ungen am normalen unbeschallten Tier (S et al, 1974) Ziel der jetzigen Untersuchur die Klärung der Fragen

1 Ändert sich nach hörschädigender S belastung die Proteinkonzentration der
2 Welche Rückschlüsse lassen sich aus solchen Konzentrationsänderung au Schädigungsmechanismus ziehen?

Die Untersuchungen wurden im Auftrage des Ministeriums fur Gesundheitswesen der DDR im Rahmen der Larmschadenforschung durchgefuhrt

MATERIAL UND METHODEN

Gewinnung der PL

Die Untersuchung wurde mit 200–300 g sch normalhorenden Meerschweinchen (Prüfur dem Preyerschen Ohrmuschelreflex) in urethan-Narkose durchgeführt Die PL-G nung erfolgte in der schon beschriebenen (Scheibe et al, 1975) unmittelbar postm und am lebenden Tier nach verschied Methoden

Beschallung der Tiere

Fur die Belastungsversuche wurden die T einseitig im akustisch geschlossenen S (Wagner et al, 1974) 1 Stunde mit Breitb rauschen der Intensität von 140 dB besc Unter einem akustisch geschlossenen S verstehen wir die schalldichte Ankopplung dynamischen Meßschallwandlers an den

abelle I *Proteinkonzentration (mg/100 ml) von ich Perfusion mit Ringer-Lösung postmortal wonnener Gesamt-Perilymphe einseitig beschallter Tiere*

rilymph Entnahme ch Beschallung	Kontralaterales Ohr	Beschalltes Ohr
fort	146±29 (12)	174±53 (12)
ich 6 Stunden	139±24 (10)	176±44 (10)
ich 1 Tag	154±40 (9)	150±39 (9)

ernen Gehorgang des Meerschweinchens mit ilfe eines Trichters Bei den angewendeten eschallungsparametern ist mit einer starken hädigung des Haarzellbestandes auf der samten Basilarmembran zu rechnen Die PL urde zu verschiedenen Zeiten nach Beschal ngsende entnommen

oteinbestimmung und Auswertung

ie Proteinbestimmung erfolgte nach Lowry al (1951) in der schon beschriebenen Mikroodifikation (Scheibe et al , 1974)

Innerhalb der einzelnen Versuchsreihen wurde e PL-Proteinkonzentration der beschallten hren mit der der kontralateralen Ohren rglichen Da die kontralateralen Ohren der nseitig beschallten Tiere nicht als unbeschallt lten konnen, erfolgte außerdem ein Vergleich rer Proteinwerte mit denen normaler unschallter Tiere (Scheibe et al, 1974) Die üfung der Mittelwerte wurde mittels *t* Test ırchgefuhrt

ERGEBNISSE

L-Gewinnung

ach Schallbelastung ist die intravitale Gewin ıng blutarmer PL, besonders aus der Scala mpani, noch wesentlich schwieriger als bei ormaltieren Die sonst vorteilhafte Entnahme r tympanalen Proben durch das runde Fenster t bei den beschallten Ohren ungünstig Das ınde Fenster erscheint makroskopisch in den eisten Fallen stark gerotet Unter dem Operaonsmikroskop erkennt man eine deutliche efaßerweiterung Dadurch läßt sich bei der Punktion der runden Fenstermembran eine Gefaßverletzung und damit eine Blutverunreinigung der PL kaum vermeiden Nach Verdünnung des Knochens ist mikroskopisch auch die Erweiterung der Gefaße an der lateralen Skalenwand zu erkennen Deshalb ist auch bei Punktion des Knochens die Ausbeute an blutarmen Proben wesentlich geringer als bei Normaltieren

Bei der postmortalen PL-Gewinnung ist ebenfalls häufiger mit Blutverunreinigung der Proben zu rechnen Nur nach intraarterieller Perfusion der Tiere ist auch von beschallten Ohren in den meisten Fällen nahezu blutfreie PL zu gewinnen

Proteinkonzentration von nach Perfusion mit Ringer Lösung postmortal gewonnener Gesamt PL beschallter Tiere

In Ergänzung unserer bisherigen qualitativen Untersuchungen (Scheibe et al , 1972) wurde auch bei den jetzigen Belastungsversuchen zunächst Gesamt PL von mit Ringer Lösung perfundierten Tieren untersucht Die PL-Proben beider Ohren (beschallt, kontralateral) wurden zu verschiedenen Zeiten nach Beschallungsende entnommen Sofort, nach 6 Stunden und nach 1 Tag Die Ergebnisse dieser 3 Gruppen sind in Tab I zusammengestellt Daraus geht hervor, daß bei den sofort (1 Reihe) und bei den nach 6 Stunden (2 Reihe) gewonnenen Proben die Proteinwerte der beschallten Ohren etwas höher liegen als bei den kontralateralen Ohren Der Konzentrationsunterschied ist (bei dem untersuchten Stichprobenumfang) aber nur bei den nach 6 Stunden entnommenen Proben signifikant ($P-5\%$) Bei den nach 1 Tag entnommenen Proben ist kein Unterschied nachweisbar

Da die PL Proteinkonzentration der kontralateralen Ohren zunächst nicht als normal, d h von der Beschallung unbeeinflußt, angesehen werden kann, haben wir diese Werte mit denen normaler unbeschallter Tiere verglichen Die am Normaltier gewonnenen Ergebnisse (Tab II) wurden in einer vorangegangenen Arbeit (Scheibe et al , 1975) bereits mitgeteilt Für die Gesamt PL fanden wir eine mittlere Proteinkonzentra

Tabelle II *Proteinkonzentration (mg/100 ml) von mit unterschiedlicher Methodik postmortal gewonnener Perilymphe unbeschallter Tiere*

Probengewinnung	Vestibuläre Perilymphe	Tympanale Perilymphe
Sofort postmortal	154±57 (27)	208±70 (15)
Nach Perfusion mit Infukoll® M 40	133±36 (16)	150±28 (13)
Nach Perfusion mit Ringer Lösung	Gesamt Perilymphe 159±40 (37)	

tion von 159±40 mg/100 ml ($n=37$) Dieser Mittelwert unterscheidet sich nicht signifikant von den Mittelwerten der kontralateralen Ohren der einseitig beschallten Tiere (Tab I) Dadurch ergeben sich auch zwischen beschalltem und unbeschalltem Ohr bzw Tier dieselben geringen Konzentrationsunterschiede wie zwischen beschalltem und kontralateralem Ohr der einseitig beschallten Tiere Wir haben deshalb auch bei den weiteren Belastungsversuchen die Tiere einseitig beschallt und zunächst das beschallte mit dem kontralateralen Ohr verglichen Zusätzlich erfolgte auch hier noch ein Vergleich mit den analytischen Daten der unbeschallten Tiere (Tab II)

Proteinkonzentration von postmortal gewonnener vestibulärer und tympanaler PL nach Schallbelastung

Bei funktionellen Untersuchungen sind Konzentrationsänderungen in der PL, die durch Stoffwechseländerung oder Zellschädigung des Cortischen Organs bedingt sein können, in stärkerem Maße in der tympanalen als in der vestibu[illegible] PL zu erwarten Die vorangegangenen Un[illegible]suchungen am Normaltier (Scheibe et al [illegible]) haben aber gezeigt, wie problematisch gerad[illegible] Gewinnung der tympanalen PL ist Auch [illegible] subokzipital eröffnetem Liquorraum [illegible] die tympanalen Proteinwerte relativ stark [illegible] den beschallten Tieren ist es außerdem [illegible]sonders schwierig, die tympanale PL intra [illegible] blutfrei zu gewinnen Wir haben deshalb [illegible] den weiteren Belastungsversuchen postmo[illegible] gewonnene PL untersucht

Die PL wurde zuerst durch die übl[illegible] Punktion der herausgebrochenen Schnec[illegible] sofort bzw 6 Stunden nach Beschallungse[illegible] gewonnen Obwohl auch bei diesen Vers[illegible] reihen nur PL mit einer Blutverunreinig[illegible] <0,25% verwendet wurde, erschienen [illegible] tympanylen Proben, und zwar sowohl von [illegible] beschallten als auch von den kontralater[illegible] Ohren, nach dem Zentrifugieren häufig et[illegible] hämolytisch Von den sofort nach der Besch[illegible]lung entnommenen tympanalen Proben wa[illegible] ungefähr 25–30% etwas hämolytisch Sie wu[illegible] nicht in die Berechnung mit einbezogen [illegible] Ergebnisse dieser Versuchsreihe sind in Tab [illegible] (1 Reihe) dargestellt Sie zeigen, daß sich [illegible] Proteinwerte der beschallten und kontralatera[illegible] Ohren unmittelbar nach der Beschallung ni[illegible] unterscheiden, weder von vestibulärer n[illegible] von tympanaler PL

Von den 6 Stunden nach Beschallungser[illegible] entnommenen tympanalen Proben (Tab [illegible] 2 Reihe) waren bei den kontralateralen Oh[illegible] ungefähr 20% etwas hämolytisch und wur[illegible] verworfen Bei den beschallten Ohren wa[illegible]

Tabelle III *Proteinkonzentration (mg/100 ml) von mit unterschiedlicher Methodik postmo[illegible] gewonnener vestibulärer und tympanaler Perilymphe einseitig beschallter Tiere*

Perilymph Entnahme nach Beschallung	Vestibuläre Perilymphe		Tympanale Perilymphe	
	Kontralaterales Ohr	Beschalltes Ohr	Kontralaterales Ohr	Beschalltes Ohr
Sofort	136±42 (21)	138±51 (21)	152±50 (13)	159±46 (11)
Nach 6 Stunden	153±37 (10)	159±38 (13)	186±75 (7)	(240±77) (8)
Nach 6 Stunden, Perfusion mit Infukoll M 40	185±42 (28)	208±56 (28)	163±42 (20)	171±51 (19)

wegen ungefähr 75 % der tympanalen Proben hämolytisch Dadurch wäre für einen genugend großen Stichprobenumfang von blutarmen nicht hämolytischen Proben ein sehr hoher Aufwand an Tiermaterial erforderlich gewesen Wir haben deshalb für eine orientierende Abschätzung die hämolytischen Proben zunächst in die Berechnung mit einbezogen Das bedeutet, daß bei einer maximalen Blutverunreinigung von 5 % die Proteinkonzentration der tympanalen Einzelwerte durch Hamolyse bis zu ungefahr mg/100 ml artefiziell erhoht sein kann Dadurch ist auch der tympanale Mittelwert (0±77) artefiziell erhöht und wurde deshalb in Tab III (2 Reihe) in Klammern gesetzt Eine Aussage über einen signifikanten Konzentrationsanstieg in der tympanalen PL der beschallten Ohren ist daher bei dieser Versuchsreihe nicht möglich Die vestibulären Proteinwerte der beschallten (159±38) und der kontralateralen Ohren (153±37) unterscheiden sich auch bei dieser Versuchsreihe nicht

Bei beiden Versuchsreihen ändert sich das Untersuchungsergebnis nicht signifikant, wenn man die Proteinwerte der beschallten Ohren mit den entsprechenden Werten der unbeschallten Tiere (Tab II, 1 Reihe) vergleicht

Proteinkonzentration von nach Perfusion mit Infukoll® M 40 postmortal gewonnener vestibulärer und tympanaler PL beschallter Tiere

Um für abschließende Belastungsversuche möglichst blutfreie PL zu erhalten, haben wir in der letzten Versuchsreihe die beschallten Tiere vor dem Dekapitieren intraarteriell mit Infukoll® M 40 perfundiert Auf Grund der bisherigen Belastungsergebnisse wurde die Untersuchung auf 6 Stunden nach Beschallungsende entnommene Proben beschrankt Trotz der Perfusion der Tiere waren ungefahr 20–30 % der tympanalen beschallten und kontralateralen Proben nach dem Zentrifugieren etwas hämolytisch bzw trub und wurden verworfen Die Proteinwerte sind in Tab III (3 Reihe) enthalten Sie zeigen, daß sich bei dieser Versuchsreihe die tympanalen Mittelwerte der beschallten (171±51) und kontralateralen Ohren (163±42) kaum unterscheiden Bei der vestibulären PL liegt dagegen die Proteinkonzentration der beschallten Ohren (208±56) etwas höher als bei den kontralateralen Ohren (185±42) und bei Paarvergleich ergibt sich Signifikanz für $P = 5$ % Auch bei dieser Versuchsreihe ändert sich die Aussage nicht, wenn man für den Vergleich wieder die Proteinwerte der unbeschallten Tiere (Tab II, 2 Reihe) heranzieht

DISKUSSION

Die analytischen Ergebnisse zeigen, daß nach stark horschädigender Schallbelastung nur geringe Konzentrationsanderungen der PL-Proteine bei den postmortal gewonnenen Proben der intraarteriell perfundierten Tiere nachweisbar sind Der Konzentrationsunterschied ist bei den 6 Stunden nach Beschallungsende entnommenen Proben am deutlichsten Hier fanden wir in der Gesamt PL der mit Ringer Lösung perfundierten Tiere (Tab I, 2 Reihe) und in der vestibularen PL der mit Infukoll® M 40 perfundierten Tiere (Tab III, 3 Reihe) bei den beschallten Ohren eine signifikant ($P = 5$ %, Paarvergleich) höhere Proteinkonzentration als bei den kontralateralen Ohren

Bei den nicht perfundierten Tieren (Tab III, 2 Reihe) ist eine Konzentrationserhöhung in der vestibularen PL der beschallten Ohren bei dem untersuchten Stichprobenumfang nicht nachweisbar Der erhohte tympanale Proteinwert der beschallten Ohren ist wahrscheinlich durch Hamolyse in den Proben bedingt Bei dieser Versuchsreihe müßte für eine eindeutige Aussage der Stichprobenumfang insgesamt erhöht werden Dazu wäre aber wegen der geringen Ausbeute an blutarmen nicht hämolytischen Proben, besonders bei der tympanalen PL der beschallten Ohren, ein sehr hoher Aufwand an Tiermaterial erforderlich

Am lebenden Tier, bei dem die Gewinnung blutfreier PL noch schwieriger ist als postmortal, sind Belastungsversuche mit größerem Stichprobenumfang (bei Schallintensitäten von 140 dB) praktisch kaum durchzuführen Hinzu kommt hier bei den tympanalen Proben die

mogliche Verfälschung durch Liquor. Nach unseren Untersuchungen am Normaltier (Scheibe et al, 1975), bei denen sich die Proteinwerte *intravital und postmortal gewonnener Proben* nicht signifikant unterscheiden, sind aber auch am lebenden Tier keine wesentlich anderen Belastungsergebnisse zu erwarten

Der Vergleich mit den Proteinwerten der unbeschallten Tiere (Tab II) zeigt, daß die PL-Proteinkonzentration der kontralateralen Ohren durch die einseitige Beschallung der Tiere nicht signifikant verändert wird Zwischen beschalltem und unbeschalltem Ohr bzw Tier ergeben sich daher auch nur dieselben geringen Konzentrationsunterschiede wie zwischen beschalltem und kontralateralem Ohr der einseitig beschallten Tiere

Eine Erklärung für die etwas hohere Proteinkonzentration in der PL der beschallten Ohren kann bisher nicht gegeben werden In Betracht käme hauptsächlich ein Proteinaustritt aus schallgeschädigten Zellen des Cortischen Organs (Spoendlin & Brun, 1973) und eine erhohte Proteinpermeabilitat der Kochleagefäße

Die Belastungsversuche an Kaninchen (Miyake, 1960) und Katzen (Komarovich & Plouzhnikov, 1966) ergaben unterschiedliche Ergebnisse Miyake berichtet lediglich, ohne Angaben von Analysenwerten und Versuchsbedingungen, daß nach Beschallung eine Abnahme der Proteinkonzentration in der PL eintritt Komarovich & Plouzhnikov haben in *2 Versuchsreihen je 6 Tiere 20–60 Minuten mit* einer Sirene (Gesamtschallpegel 140 dB, Grundschwingung 400 Hz) belastet und die PL 30 Minuten bzw 3 Stunden nach Beschallungsende untersucht Die Proteinwerte lagen bei Entnahme nach 30 Minuten niedriger und bei Entnahme nach 4 Stunden höher als bei unbeschallten Kontrolltieren Statistisch gesichert wurden diese Angaben nicht Als Ursache der Konzentrationsabnahme wird eine Permeabilitätsänderung der Reißnerschen Membran vermutet Die Konzentrationserhöhung wird als Folge eines nachfolgenden kompensatorischen Proteinaustrittes aus Kochleagefaßen angesehen

Die vorliegenden Ergebnisse bestätigen unsere fruher geäußerte Annahme (Scheibe et al, l daß es bei funktionellen Untersuch schwierig ist, geringe Konzentrationsänder *in der PL eindeutig festzustellen* Blu Liquorverunreinigung der PL sowie individuelle Links-Rechts-Unterschiede de teinwerte machen selbst bei einseitiger Bes lung der Tiere und paarweisem Vergleich beschallten und unbeschallten Ohren einde Aussagen über geringe Konzentrations rungen der PL-Proteine problematisch E daher auch verständlich, daß bei uns früheren immunelektrophoretischen Ur chungen (Scheibe et al, 1972) nach Schall stung keine Änderung der PL-Proteine r weisbar war

Zusammenfassend lassen sich die als Zie lung formulierten Fragen wie folgt beantwo

1 Selbst stark hörschädigende Schallbelas verursacht nur geringe Konzentrationsä rungen der PL-Proteine Ihr eindeu Nachweis ist problematisch
2 Rückschlüsse auf einen Schallschädigu mechanismus konnen aus den Ergebni nicht gezogen werden

SUMMARY

The protein concentration in the perilymph of n exposed guinea pigs was investigated using a method described previously The animals were ex to wide band noise at 140 dB for one hour unilat in a closed acoustic system Perilymph was obtained exposure at different intervals immediately, 6 c *hours later From animals exposed to noise, it is p* ularly more difficult to obtain perilymph without b contamination than from normal ones Even post mo it is difficult to obtain samples without blood conta tion and without hemolysis particularly from the tympani Therefore, some animals were perfused i arterially with Ringer solution or with Infukoll before decapitation *After exposure to noise only a* increase in the protein concentration of the peri can be detected in the noise-exposed ears of a perfused intra arterially The difference in concent is most distinct in samples extracted 6 hours after exposure had ceased No reliable clue to the sour this protein could be obtained from these investig

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Dr rer nat F Scheibe
HNO Klinik der Humboldt Universitat
(Charité)
Schumannstr 20–21
104 Berlin, DDR

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VERBINDUNGSKOMPLEXE AN ZELLEN DER REISSNER-MEMBRAN IN GEFRIERGEBROCHENEN PRÄPARATEN*

K D Franke[1], E Reale[2], S Iurato[3], L Luciano[2], G Wermbter[2] und E Pannese[4]

Aus der [1]Hals Nasen Ohrenklinik und der [2]Abteilung fur Elektronenmikroskopie des Anatomischen Instituts der Medizinischen Hochschule Hannover, BRD, dem [3]Institut fur Bioakustik der Universitat Bari und dem [4]zweiten Anatomischen Institut der Universitat Mailand, Italien

(Eingegangen am 20 September, 1974)

Abstrakt Die Reissner Membran aus dem Innenohr von Chinchillas wurde mit Hilfe der Gefrieratzungsmethode untersucht Zonulae occludentes schließen endolymph warts den Raum zwischen den Epithelzellen ab Sie bestehen aus 2 bis 8 ubereinanderliegenden Kammen bzw Talern und ähneln somit morphologisch denen zwischen den Marginalzellen der Stria vascularis, sind jedoch wesentlich schwacher ausgebildet als die zwischen den Basalzellen der Stria

Die Endolymphe unterscheidet sich von der Perilymphe chemisch durch einen höheren [K^+]- und niedrigeren [Na^+] Gehalt (Smith et al, 1954, Johnstone et al, 1963), elektrophysiologisch ist ein hoheres positives Potential der Scala media gegenüber der Scala vestibuli bzw tympani meßbar (v Bekesy, 1952, Johnstone, 1967)

Hohe Konzentrations- und Potentialunterschiede werden von Epithelien mit physiologisch dichten Verbindungskomplexen, sogenannten „tight junctions“, aufrechterhalten (Frömter & Diamond, 1972) Claude & Goodenough (1973) fanden den morphologischen Aspekt der „tight junctions“, wie er in Abdrücken gefriergebrochener Präparate darstellbar ist, korrelierbar mit dem elektrischen Widerstand des jew Epithelverbandes In dieser Hinsicht habe die Reissner-Membran mit der Gefr methode untersucht

MATERIAL UND METHODE

Sieben erwachsene Chinchillas wurden n mg/kg Natrium-Pentobarbital endoperi anaesthesiert und die Innenohren durch lymphatische Perfusion vorfixiert Danacl lierten wir die Cochlea, entfernten den S und setzten die Fixierung für weitere vier St fort Während dieser Zeit haben wir die cherne Kapsel der Cochlea entfernt, den D cochlearis abgetrennt und in einem Fall - eine gezielte Präparation — die Rei Membran isoliert Die Fixierungslösung en 2 % Formaldehyd und 1 % Glutaraldehyd i M Cacodylatpuffer (pH 7,3 — 7,4) mit 25 $CaCl_2$ Nach beendeter Fixierung wurden Präparate 90 Minuten mit 30 % Glyzeri Ringerlosung durchtrankt, auf Präparatträ plättchen orientiert, in flüssigem Freon eingefroren und in einem Balzers Gerat B M gebrochen sowie mit Platin Kohle bed (Moor & Mühlethaler, 1963) Die Abd reinigten wir in Chromsaure und Natriu pochlorit, fingen sie auf Dowell-Membr auf und untersuchten sie in Siemens Elektr mikroskopen Ia und 101

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* Vorgetragen auf der 45 Jahresversammlung der Deutschen Gesellschaft fur Hals Nasen Ohren Heilkunde Kopf und Hals Chirurgie (Bad Reichenhall 26 - 30 5 1974)

Mit Unterstutzung des Consiglio Nazionale delle Ricerche' (Nr CT 72.00775 04 115 2277 und 71 00863 04 115 1144)

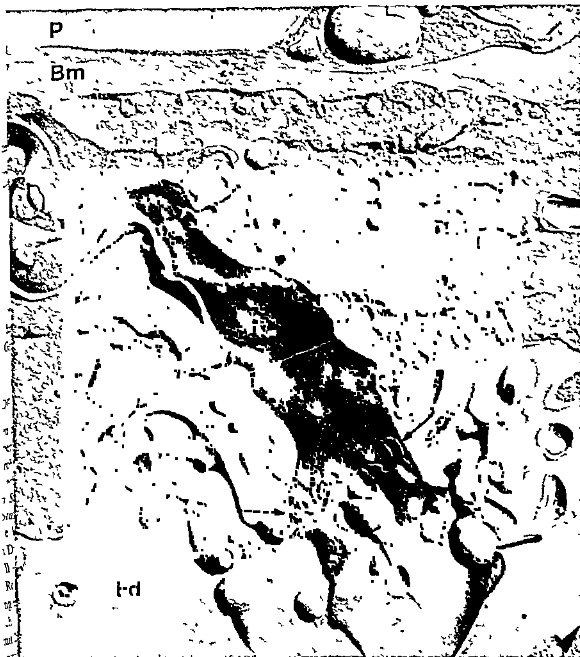


Abb 1 Abdruck einer gefriergebrochenen Reissner Membran *P* = Perilymphraum *Bm* = Basalmembran, *Ed* Endolymphraum. Die Pfeile weisen auf die Zonula occludens der Epithelzellen Die Pfeilspitze gibt die Bedampfungsrichtung an × 50 000

ERGEBNISSE

Die Abdrucke der gefriergebrochenen Präparate der Reissner-Membran lassen die unter anderem am Meerschweinchen im Dunnschnitt von Hagiwara (1963) Iurato (1967*a*, *b*), Duvall & Rhodes (1967), v Ilberg (1968) und Duvall & Sutherland (1970) beschriebenen Strukturen erkennen

Die beiden Zellschichten ektodermaler und mesenchymaler Herkunft sind beim Chinchilla deutlich sichtbar (Abb 1) Die endolymphseitig

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legenen Epithelzellen bilden eine vollständige cht, die auf der nur teilweise erkennbaren an liegt Sie tragen kurze Mikrovilli d zeigen im Zytoplasma einen ausgedehnten olgi Apparat, Mitochondrien und Zisternen des doplasmatischen Retikulums, vorwiegend ıßalwärts liegen zahlreiche Mikropinozytose esikeln Die Plasmamembranen benachbarter llen verlaufen unmittelbar unterhalb des rbindungskomplexes meistens geradlinig, wer n dann leicht gewunden und bilden schließlich der Nahe der Basalmembran Falten

Im Bereich des Verbindungskomplexes sind amme (Flache A der Zellmembran) und/ er Taler (Fläche B der Zellmembran) sichtbar sind miteinander anastomosiert und bilden i unregelmäßiges Netzwerk (Abb 1 und 2) Kamme und Täler stellen Kontaktlinien eier benachbarter Zellmembranen dar Mor ologisch entsprechen sie der Zonula occludens, e im gleichen Bereich anderer Epithelien monstriert wurde (Kreutziger, 1968, Staehelin al, 1969, Chalcroft & Bullivant, 1970, iend & Gilula, 1972, Claude & Goodenough, 73)

Die Zahl der Kamme bzw Täler variiert ischen zwei (Abb 1) und acht (Abb 2*d*) Sogenannte „gap junctions" konnten wir lang im Epithel der Reissnerschen Membran ht finden

Die bindegewebigen, flachen Zellen der peri ophwärts gelegenen Schicht der Reissner mbran lassen auch in Abdrücken gefrier rochener Präparate häufig weite Unter chungen erkennen Infolgedessen ist eine nula occludens hier nicht zu erwarten „Gap ctions", die an den Zellen gleicher Herkunft Ligamentum spirale zahlreich vorkommen rato et al, 1974), waren in unseren Präparaten her nicht sichtbar

DISKUSSION

ntsprechend den Beobachtungen an Dünn nitten zeigen unsere Untersuchungen an drucken gefriergebrochener Praparate der ssner-Membran, daß endolymphwärts der Spalt zwischen den Epithelzellen durch Zonulae occludentes abgeschlossen wird

Die Zonulae occludentes können nach physiko chemischen und morphologischen Kriterien in „leaky" und „tight" unterteilt werden Verbände von Epithelzellen mit undurchlässigen („tight") Zonulae occludentes weisen nach Frömter & Diamond (1972) u a einen transepithelialen elektrischen Widerstand von 365 bis 2 000 Ohm cm² auf, ihr Ruhepotential beträgt 30 bis 100 mV Bei Claude & Goodenough (1973) werden in Abdrucken gefriergebrochener Epithelien mit einem transepithelialen elektrischen Widerstand von 300 bis 600 Ohm cm² die Zonulae occludentes als aus zwei bis sieben ubereinander liegenden Kammen, bzw Tälern, zusammengesetzt beschrieben und als „intermediate to tight" klassifiziert Diese Werte werden für die „leaky junctions" wesentlich niedriger angegeben

Es stellt sich die Frage, ob die Zonulae occludentes des Epithels der Reissner-Membran zum „tight" oder zum „leaky" Typ gehoren, d h ob sie an der Aufrechterhaltung des hohen $[K^+]$ Gehaltes der Endolymphe und des endocochlearen Potentials beteiligt sind Der trans epitheliale elektrische Widerstand dieser Membran beträgt 368 Ohm cm² (Meerschweinchen, Johnstone et al, 1966), das Ruhepotential 30 bis 60 mV (Meerschweinchen, v Ilberg & Imamura, 1966), die Zahl der Kamme bzw Taler der Zonula occludens nach unseren Untersuchungen beim Chinchilla meistens sechs oder mehr, aber stellenweise auch zwei

Die Barriere zwischen Endolymphe und Perilymphe an der Reissner Membran kann deshalb unter Heranziehung der oben angegebenen Werte für Meerschweinchen und Chinchilla dem Typ „tight" nach Fromter & Diamond (1972) und „intermediate to tight" nach Claude & Goodenough (1973) zugeordnet werden

Die Zonulae occludentes der Reissner-Membran ahneln morphologisch denen der Marginalzellen der Stria vascularis, dagegen weisen sie wesentlich weniger Kämme bzw Täler auf als z B die Zonulae occludentes zwischen den Basalzellen der Stria vascularis (Iurato et al, 1974)

SUMMARY

The Reissner membrane of the chinchilla inner ear was studied with the freeze-fracture method Zonulae occludentes, composed of 2 to 8 strands, seal the intercellular space close to the endolymphatic surface They are morphologically similar to those seen between the marginal cells of the stria vascularis, but much less developed than those between the basal strial cells

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Prof E Reale
Anatomisches Institut
Abt Elektronenmikroskopie
Medizinische Hochschule
Karl Wiechert Allee 9
3 Hannover, BRD

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AN ELECTRON MICROSCOPIC STUDY OF THE OTOLITHIC MACULAE OF THE LAMPREY (*ENTOSPHENUS JAPONICUS*)

T Hoshino

From the Department of Otolaryngology, Teikyo University School of Medicine, Tokyo Japan

(Received August 14, 1974)

Abstract The sensory epithelium of the otolithic maculae om the inner ear of the lamprey (*Entosphenus japonicus*) as studied under the scanning and transmission electron icroscope Two different types of sensory hair cells re discerned and each had a patterned distribution

iated organelles were found in the cytoplasm of both pes of sensory cells These striated organelles exhibited riodic electron-dense and less dense striations of 170 μm tending from the cuticular plate down to the basal rtion of the cell Generally this organelle was found profusion in the sensory cells with long hair bundles it rarely found in the other type of cells

ylogenetically, the inner ear of the lamprey in a unique position in that it has only two micircular canals instead of the three canals und in other gnathostomes Because the lam ey labyrinth is considered to be a prototype the vertebrate labyrinth, detailed studies of e lamprey have been made using both light icroscope and electron microscope (de Burlet Versteegh, 1930, Lowenstein et al 1968, owenstein & Thornhill, 1970, Thornhill, 1972) Physiologically, the lamprey labyrinth has en shown to be capable of the whole range of sponses characteristically found in the laby ith of the higher vertebrates (Löwenstein, 70) These responses were in the form of ural activity recorded during angular accelera- ons in various planes, including the horizontal ane, and recordings of positional and vibra- onal responses

is study was partly supported by U S P H S Research ant No 10412-02

It was suggested that the organs responsible for the perception of vibration in the lamprey were localized in the vertical and dorsal maculae Subsequent to these physiological findings, Lowenstein described a new type of sensory cell characterized by the presence of a long kinocilium and extremely short stereocilia, located preponderantly in the vertical and dorsal maculae Perception of vibration in the lamprey was mentioned to be related to the presence of these specialized cells in the labyrinth

This current study was undertaken in an attempt to further elucidate the different types of sensory cells found on the otolithic maculae of the lamprey (*Entosphenus japonicus*) To accomplish this histological study of the maculae, both a scanning (SEM) and a transmission electron microscope (TEM) were employed

MATERIALS AND METHODS

Adult lampreys (*Entosphenus japonicus*) with a cephalocaudal length of between 40 and 50 cm were decapitated and the cartilaginous capsule of the inner ear was removed A 2% phosphate buffered glutaraldehyde solution (pH 7 3) was gently perfused through an opening made in the upper pole of the capsule The specimens were then submerged in the glutaraldehyde solution (1 to 2 hours for TEM and 2 to 4 hours for SEM preparation) Post fixation was then applied by soaking the specimens for both TEM and SEM study in a 1% phosphate buffered osmic acid solution for one hour

SUMMARY

The Reissner membrane of the chinchilla inner ear was studied with the freeze-fracture method Zonulae occludentes, composed of 2 to 8 strands, seal the intercellular space close to the endolymphatic surface They are morphologically similar to those seen between the marginal cells of the stria vascularis, but much less developed than those between the basal strial cells

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g 2 Two distinctly different types of sensory hair indles can be seen The type A bundles (*A*) have 30 to stereocilia of graded heights (organ pipe configura on) whereas type B bundles (*B*) have stereocilia of uniform lengths about 1 μm and a long kinocilium. (*A* anterior horizontal macula *B* posterior horizontal macula) (×5 600)

3 A portion of the anterior horizontal macula owing the distribution of type A sensory ha r bundles) over the right side and in a narrow upper and lower band of the photograph and the type B sensory hair bundles (*B*) over the remaining left side of the photograph (posterior) (×380)

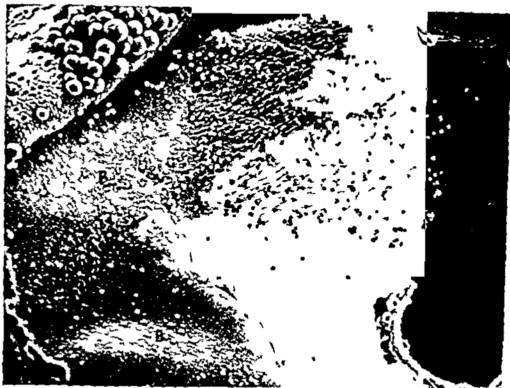


Fig 4 Low power view of the vertical macula The *anterior portion of this macula is populated with the* type A sensory hair bundles (*A*) The posterior aspect of this region tapers into a narrow band (*arro* posterior to this point the type *B* sensory hair (*B*) are found (×250)



Fig 5 A view of the entire posterior horizontal macula showing a narrow extremely lateral distribution of the type A sensory hair bundles (*A*) The remainder of the macula is occupied by type B sensory hair b× 350)



6 A portion of the vertical macula showing both the A and the type B sensory hair bundles The two s of sensory hair bundles are morphologically oppositely polarized in this picture of the vertical macula (*arrows*) (×3 660)

eocilia of approximately uniform heights 1 μm) A very long kinocilium was found one end of this bundle of stereocilia and it distinctly evident

here was a noteworthy patterned distribution hese two types of hair cells over the surface ach of the maculae observed (Figs 3–5) The A cells were found in four distinct areas the anterior half of the anterior horizontal ula with a thin lateral band running posteri- to the level of the macula sacculi, over the re surface of the macula sacculi, on the rior portion of the vertical macula with a terior tapering (Figs 4, 6), and over a small ion of the lateral edge of the posterior zontal macula The remaining segments of sensory epithelium of the otolithic maculae populated with type B cells (Fig 7)

he morphological polarization of the sensory s over the various maculae was not exten- ly observed in this study, however, a clear- polarization was seen on the vertical macula 6)

The inner structures of both the type A cells from the anterior horizontal macula and the type B cells from the posterior horizontal macula were examined under the TEM Both types of hair cells shared the following characteristics the cell bodies were cylindrical with an ovoid nucleus in the lower portion of the cell, the sensory cell layer was separated from the base- ment membrane by a supporting cell layer, the cytoplasm of the sensory cells contained many

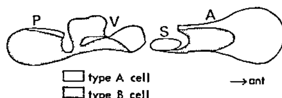


Fig 7 A schematic drawing illustrating the distribution of the type A and the type B sensory cells over the oto- lithic maculae of the lamprey (dorsal macula not shown) The vertical macula was rotated 90 degrees to illustrate it in the same horizontal plane of the other maculae *A* anterior horizontal macula *S* macula sacculi *V* vertical macula *P* posterior horizontal macula



Fig 8 Transmission electron micrograph of type A cells from the anterior portion of the anterior or horizontal macula Branching striated organelles can be most of the cells (arrows) (×5 100)

small vesicles and granules and Golgi apparatus and endoplasmic reticulum were also seen

The differences between the type A and type B sensory cells were also found in the cytoplasm of the cells Type A cells (Fig 8) presented a lower electron density of the cytoplasm due to *the reduced amount of granules and vesicles* relative to the type B cells (Fig 9) Also struc tures referred to as striated organelles were found in the cytoplasm of a majority of the type A cells but were absent in most of the type B cells These organelles appeared as which traversed the length of the cell from a point just beneath the cuticular passing close to the nucleus and several times in various directions T its course the organelle appeared to be *due to the presence of evenly spaced* bands resulting in regular alternations in densities of 170 μm Higher magnificat 10) suggested that these organelles composed of an accumulation of th



Fig 9 Transmission electron micrograph of the type B cells (*SE*) from the posterior horizontal macula. The cytoplasm exhibits a higher electron density than that of the type A cells shown in Fig 8 Striated organelles are not seen in the cytoplasm of these type B cells *SU* supporting cell (× 3 300)

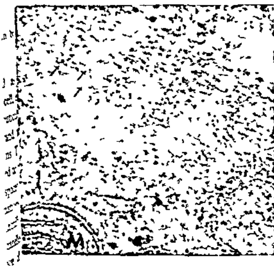


Fig 10 Higher magnification photograph of the striated organelle The organelle seems to be composed of an accumulation of thin tubular structures (*arrows*) *M* mitochondria (× 60 000)

plasmic reticulum Only a few of the type B cells from the posterior horizontal macula contained this striated organelle However, when this structure was seen in a type B cell it appeared to be much less developed and it was usually localized in the upper portion of the cell, i e, the subcuticular or supranuclear region

The sensory cells were innervated, at their bases, by means of two distinct types of nerve endings (Fig 11) One nerve ending type contained within it many mitochondria and only a few vesicles (Fig 11-I) In the adjacent sensory



Fig 11 The different types of nerve endings seen at the base of the sensory cell 1 A nerve ending containing many mitochondria (*M*) and only a few vesicles A round electron-dense body surrounded by layers of vesicles can be seen in the sensory cell body (*SE*) This is assumed to be a synaptic body accompanying an afferent ending (×67 000) 2 A nerve ending containing vesicles A subsynaptic cistern (*arrow*) can be seen in sensory cell body (*SE*) close to the synaptic mem- *SU* supporting cell (×30 000)



Fig 12 Unmyelinated nerve fibers running beneath the posterior horizontal macula Marked differences in the diameters of these fibers can be seen. *G* ganglion (×3 500)

l a homogeneous dense body (synaptic body) s frequently found located near the thickened naptic membrane and surrounded by layers of icles The other type of nerve ending exhibited a ckened membrane on both the nerve ending d on the adjacent sensory cell body As posed to the first type of nerve ending scribed above, this second type (Fig 11-2) ntained many small vesicles (with diameters 60 to 70 μm), a few larger cored vesicles 0 to 120 μm in diameter) and a few mito- ondria Also, a sub-synaptic cistern was seen the second type of nerve ending (arrow, g 11-2)

No distinct pattern was discerned relative to innervation of the type A and the type B nsory cells by these two different types of nerve dings Judging from the results from previous estigators regarding sensory endorgan syn- ses, it seems likely that these two types of ve endings correspond to the afferent (Fig -1) and efferent (Fig 11-2) endings

The nerve fibers which course beneath the terior and the posterior horizontal maculae re found to be all unmyelinated However, re was wide variation in the diameters of these ers (Fig 12)

COMMENTS

the older literature (de Burlet & Versteegh, 30) the nomenclature of the otolithic maculae the lamprey was based on the homology of gnathostomes However, Lowenstein et al 68) recently advocated a revision of this menclature based upon a simpler topo- phical view of the lamprey labyrinth In this rent report, Lowenstein's revision has been opted with the exception of the macula culi for which the older classical term was d In the present study, the polarization of the sory cell cilia was not extensively observed l thus further ideas regarding classification l nomenclature have not been tendered

Sensory cells bearing uniformly short stereo- a and a long kinocilium had previously been cussed by Lowenstein et al (1968) These cells were found predominantly on the vertical and dorsal maculae, the maculae from which the responses to vibration were electrophysio- logically recorded by the same author (Löwen- stein, 1970) However, in the current study, this particular type of sensory cell (type B) was to be confined not only to the vertical and dorsal maculae They were also distributed rather profusely over the posterior segment of the macula anterior horizontal and also over most of the posterior horizontal macula This discrepancy is most likely based on the fact that Löwenstein et al (1968) described the otolithic maculae of the *Lampetra fluviatilis* whereas this present study examined the maculae of the *Entosphenus japonicus* Regardless, the results of this current study suggest that the perception of vibration in the lamprey cannot be solely attributed to the hair cells with short stereocilia and a long kinocilium (the type B cells)

The striated organelle found within the labyrinthine sensory cells of the ammocoete larva of the lamprey was first described by Löwenstein & Osborne (1964) However, these authors did not discuss the major internal differences in structure amongst the cells which they examined In the current study it could be seen that the development of the striated or- ganelle was more complete in the type A cell relative to the type B cell

Similar striated structures have been dis- cerned in sensory cells taken from pathological otolithic maculae, i e, from patients with Meniere s disease (Friedmann et al, 1963) and from animals treated with ototoxic drugs (Jahnke 1969) These intracellular striated structures have also been found in non labyrin thine cells taken from non pathological speci mens, i e, from the tympanic muscles of the cat (Hirayama & Daly, 1974), in the extra ocular muscles of humans (Mukuno, 1966), in the neurons of the geniculate nucleus of the cat (Smith et al, 1964), in the lateral geniculate body neurons of the cat (Morales et al, 1964) and at the receptor synapses of the guinea pig retina (Mountford, 1964)

Striated organelles found in sensory cells

from pathological labyrinthine sensory epithelium (i e, Menière's disease or experimental ototoxicity) show a very thin transverse line located between each of the regularly spaced electron-dense bands Also, in these pathological cells, the striated organelle is found only in the immediate sub cuticular area of the cell Striated organelles in normal sensory cell, as reported in the study, did not exhibit this intermediate transverse line Higher magnification views of the electron-dense bands on normal sensory cell organelles indicated that these bands were actually composed of closely spaced, longitudinally oriented, tubular structures probably consisting of differentiated endoplasmic reticulum Although a stimulus conducting role or even an intracellular support role could be attributed to these striated organelles, no particular conclusion could be offered concerning their function Thus the differential distribution of these organelles in the type A and the type B cells sheds little light on their possible functional differences

It is felt that the observation of two different types of sensory cells in the otolithic maculae of the lamprey is rather noteworthy Although many differences regarding the morphology ' the nerve endings and the general shape of the sory cells from the mammalian otolithic maculae have been published, there has been little discussion regarding distinct differences between the sensory hairs or between the specific intracellular organelles In a few SEM studies of the otolithic maculae of fish and amphibians (Arenberg & Rauchbach, 1973, Lewis & Li, 1973), differences in the lengths of the sensory hairs on cells from different maculae were discussed However, these reports did not show a clear-cut dichotomy between the hair cell bundle types or between the presence of striated organelles in the sensory cell bodies as found in the lamprey It seems likely that further physiological studies of vibration and equilibration perception will elucidate functional differences underlying the morphological differences between the two types of sensory cells from the otolithic maculae of the lamprey

ACKNOWLEDGEMENTS

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ZUSAMMENFASSUNG

Die morphologischen Einzelheiten des Sinne[illegible] vom Lampreteninnenohr wurden im Elektrone[illegible] skop und Rasterelektronenmikroskop untersuch[illegible] Arten von Haarzellen mit verschiedenen Sinne[illegible] wurden in der Macula gefunden. Der eine Ze[illegible] hatte 20-30 kurze Stereozilien und ein langes Kin[illegible] an der Oberfläche Der andere Typ hatte noch [illegible] Stereozilien mit stufenweise erhohter Haarlä[illegible] des einen Seite des Zellen bis zu anderen Dies[illegible] zellen zeigten sogenannte „striated organelle" i[illegible] plasma Im allgemeinen wurden diese Organelle [illegible] haltig in den Sinneszellen mit den langen Haart[illegible] dagegen im anderen Zellentyp kaum gefunden [illegible] verschiedenen Haarzellen wiesen eine bestimm[illegible] teilung in jeder Macula des Lampreteninnenoh[illegible]

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T Hoshino, M D
Dept of Otolaryngology
Teikyo University School of Medicine
Kaga 2 11-1, Itabashi Ku,
Tokyo 173
Japan

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METHODOLOGIC OBSERVATIONS ON TYMPANOMETRY WITH REGARD TO THE PROBE TONE FREQUENCY

V Colletti

From the 2nd ENT Department, University of Padua, Verona, Italy

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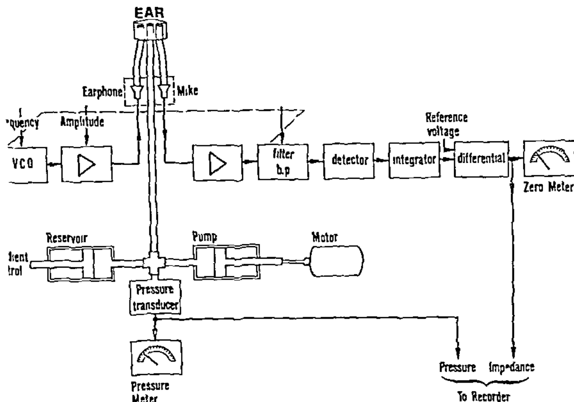
Abstract Changes in the *tympanogram shape* in the frequency range from 200 to 2 000 Hz have been investigated The tympanogram undergoes, as the probe tone frequency rises, characteristic *morphologic* modifications which are consistently observed both in normals and in some middle ear disorders The only exception to this regular behavior is the pathological picture in which the structure or the dynamic of the tympano ossicular system is remarkably anomalous (cholesteatoma and otitis media) where there are flat tympanograms It is suggested that *the frequency interval* at which one of the characteristic tympanometric configurations appears is *the element capable of furnishing a diagnostic tool* for differentiating pathophysiologic conditions of the middle ear

It is known from many investigations that a difference in the static pressure on the two sides ' the tympanic membrane change the acoustic , (functions) of the middle ear and consequently produce recordable changes in the ear's acoustic impedance (Thomsen, 1955, Anderson et al, 1956, Therkildsen & Thomsen, 1959, Møller, 1965)

On the basis of the above-mentioned experimental findings, a clinical method known as tympanometry has emerged for assessing the ear drum mobility, the status of the ossicular chain, the middle ear pressure and the Eustachian tube function (Brooks, 1968, 1969, Lidén et al, 1970, Jerger, 1970, Olivier, 1971, Bel et al, 1972, Marquet et al, 1973, Van den Eeckout et al, 1973) Tympanometry consists, in short, in measuring the ear's acoustic impedance as a function of an externally applied air pressure in the ear canal While the validity of tympanometry in assessing and discriminating among various middle ear pathologic conditions been demonstrated by several of these p there is still controversy as to what const the most effective probe tone frequency recent literature reports two main opposing of opinions and experience

Alberti & Jerger (1974), in a compa analysis of the tympanograms obtained probe tone frequencies of 220 and 800 various types of middle ear disorders, asser the occurrence of a W pattern observable a Hz, seems most closely related to diseas the tympanic membrane but, unfortunatel W pattern may also be a normal variant T fore the authors concluded that "there doe appear to be substantial clinical value in cuting tympanograms at probe tone freque higher than 220 Hz" Lidén et al (1974), o contrary, stress that in order to gain the valuable information for the differential dia sis of conductive impairments, the tymp metric investigation should be carried out probe tone frequency of 800 Hz, a frequ within the resonance frequency range of human middle ear

In a preliminary report, Colletti (1974) cussed the methodologic reasons for us probe tone of variable frequencies (200 to Hz) The present investigation concerns relation between the pattern of the tym gram and the frequency of the probe tone alteration of the shape of the tympanogr *a function of various middle ear patholo*



1 Block diagram of the equipment used in multi
quency tympanometry

o discussed The relation between the tym hogram and the intensity of the probe tone well as the static pressure, to be imposed in a ear canal, will be reported in a subsequent ublication (Colletti, in preparation)

METHOD

e basic equipment that we now routinely use tympanometry is shown in the block diagram Fig 1 The reader is referred to a previous per for technical details (Colletti, 1974) The canal and the tympanic membrane of the tients were examined before the initiation of npanometry Tympanograms were measured st at 200 Hz and then repeated with 200 Hz rements up to 2 000 Hz the highest frequency ted When a new tympanometric shape appeared in the investigation the frequency reso ion was increased, and steps of 20 Hz were d Normally the pressure was kept between −200 and −200 mmH$_2$O If the impedance/pressure function revealed a flat or flat-descend ing slope, the range of pressure values was in creased to ±400 mmH$_2$O The rate of change of the pressure gradient was kept at 10 mmH$_2$O/sec and the sound level was below the individual threshold of the stapedius reflex The output signals from the pressure transducer and the electroacoustic bridge were recorded on a two channel recorder (Electronystagmograph Mod Galileo) The air pressure in the ear canal, and the corresponding impedance value could thus be observed simultaneously

SUBJECTS

Two groups of subjects will be reported in the present study The first group consisted of nor mal hearing subjects and included twenty ears The subjects selected were medical students Ears with progressive or actual pathology were

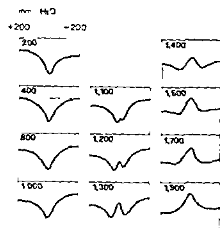


Fig 2 Tympanograms at several probe tone frequencies obtained in a normal hearing subject with normal tympanic membrane for decreasing pressure values (from +200 to −200 mmH$_2$O) at 10 mmH$_2$O/sec pressure gradient The tympanograms have been subdivided in three groups according to the morphologic similarities of the impendance/pressure functions versus frequency

excluded from our test procedures Pure tone audiograms and stapedius and tensor reflex examinations furnished evidence of normal hearing levels and normal middle ear status

The second group comprised a selection of various middle ear pathophysiologic conditions and included twenty pure otosclerotic ears, i e not complicated by progressive flogistic ep- ·es eleven successfully stapedectomized ears otosclerotic fixation of the stapedio ovalar ·on, six ossicular discontinuities, three post-myringoplastic ears, two ears with attic cholesteatoma with intact ear drum and finally ten cases of serous otitis media in the acute phase of the disease process

RESULTS

In patients with normal middle ear function, the shape of the tympanogram varies in a systematic way with the probe tone frequency The depth of the simple tympanograms, however, shows a great individual variability All subjects showed these characteristic shapes of the tympanograms which corresponded to three different ranges of probe tone frequencies Fig 2 is an example of typical tympanograms in a subject with normal hearing and normal ear drum The three patterns are clearly seen and ca· acterized as follows

(1) For low frequencies the ty· ·· are V-shaped, thus showing a single ·· with symmetrical tails

(2) With mid-range frequencies (·· ly 1100–1300 Hz) the tympanogram ·· gradual evolution to a W shape

(3) At higher frequencies (above 1·· tympanogram assumes an inverted V ·· with a single maximum and symmetrical·

The primary concern of the investigati· to determine the frequency range of each · Therefore the absolute impedance will reported In most of the pathologies (ot· sis, post-stapedectomy, ossicular disarti· and post-myringoplasty) the three · tympanogram are found The transition · patterns, however, occurs at a differen· tone frequency range, as may be obse· Fig 3 If we simply note at what fre· the W shape occurs, the following relati· observed

(1) Normal subjects, the W pattern · served at frequencies between 650 and 1·

(2) Otosclerotic ears, the emergence o· pattern occurs at higher frequencies, i e 1650

(3) Post-stapedectomized and ossicul· continuity present a W configuration in · quency range from 500 to 1000 Hz The· bution of the single ranges in this group a very limited overlapping with the norm·

(4) The post-myringoplastics' frequenc· is within the lower and upper boundaries normals, i e from 1000 to 1400 Hz

An example of the transition from V t· W to inverted V pattern is given, in de· Fig 4

Two exceptions to this frequency-dep· tympanometric pattern are seen in serou· media and cholesteatoma

The cholesteatoma cases manifested a fl· for low frequencies which evolved to a W p· above 500 Hz and finally inverted at 9·· An example is shown in Fig 5

PLURIFREQUENCY TYMPANOMETRIC INVESTIGATION

RANGE OF EXPLORED FREQUENCIES

Examined ears 72 — 200 400 600 800 1,000 1,200 1,400 1,600 1,800 2 000 Hz

NORMAL MIDDLE EAR 20

OTOSCLEROSIS 20

POST STAPEDECTOMY 11

OSSICULAR DISCONTINUITY
post traumatic 2
post flogistic 4

POST MYRINGOPLASTIC 3

ATTIC CHOLESTEATOMA 2

SEROUS OTITIS MEDIA 10

LEGEND
1st range = V shape tympanogram
2nd " = W " "
3rd " = Λ " "
flat tympanogram

Fig 3 Plurifrequency tympanometric investigation on 72

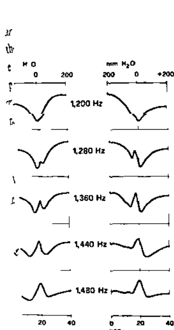


Fig 4 Dynamic of the tympanogram shape inversion The frequency range from 1 200 to 1 480 Hz is reported both for decreasing (*left side*) and increasing (*right side*) air pressure values The pressure gradient is kept constant at 10 mmH_2O sec Significant morphologic differences are appreciable as a function of the pressure direction The tympanogram configuration is reversed in respect to the horizontal axis in an interval of approximately 300 Hz.

The serous otitis media, on the other hand, displayed a flat tympanogram at all frequencies tested even with maximum pressure excursions of ± 400 mmH_2O

DISCUSSION

The present investigation suggests that the single tympanometric configuration observed by different researchers at 220, 660 and 800 Hz (quoted in the introduction) are simply typical, and single

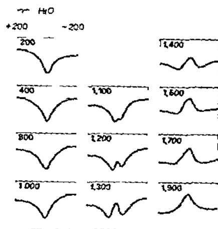


Fig 2 Tympanograms at several probe tone frequencies obtained in a normal hearing subject with normal tympanic membrane for decreasing pressure values (from +200 to −200 mmH_2O) at 10 mmH_2O sec pressure gradient. The tympanograms have been subdivided in three groups according to the morphologic similarities of the impendance/pressure functions versus frequency

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(1) For low frequencies the tympanograms are V-shaped, thus showing a single minimum with symmetrical tails

(2) With mid-range frequencies (approximately 1100–1300 Hz) the tympanogram shows a gradual evolution to a W shape

(3) At higher frequencies (above 1300 Hz) the tympanogram assumes an inverted V shape with a single maximum and symmetrical tails

The primary concern of the investigation was to determine the frequency range of each pattern. Therefore the absolute impedance will not be reported In most of the pathologies (otosclerosis, post-stapedectomy, ossicular disarticulation and post-myringoplasty) the three classes of tympanogram are found The transition between patterns, however, occurs at a different probe tone frequency range, as may be seen in Fig 3 If we simply note at what frequency the W shape occurs, the following relations are observed

(1) Normal subjects, the W pattern was observed at frequencies between 650 and 1400

(2) Otosclerotic ears, the emergence of the W pattern occurs at higher frequencies, i e 1650

(3) Post stapedectomized and ossicular discontinuity present a W configuration in the frequency range from 500 to 1000 Hz The distribution of the single ranges in this group shows a very limited overlapping with the normal

(4) The post-myringoplastics' frequency range is within the lower and upper boundaries of the normals, i e from 1000 to 1400 Hz

An example of the transition from V through W to inverted V pattern is given, in detail, in Fig 4

Two exceptions to this frequency-dependent tympanometric pattern are seen in serous otitis media and cholesteatoma

The cholesteatoma cases manifested a flat pattern for low frequencies which evolved to a W pattern above 500 Hz and finally inverted at 9[illegible] An example is shown in Fig 5

;mbrane produces a flat tympanogram at low •quency range, which evolved to smooth W 1 later inverted V at higher frequencies (actual tordings from a patient are reported in Fig 5) -s situation could not have been discriminated _n other conditions such as serous otitis media nassive tympanosclerosis on the basis of the npanogram if the analysis had been limited ´220 Hz In fact, only over 500 Hz did the ipanometric configuration assume the second ge and, very preliminarily, the third range ra possible result of an increase of the mass nponent of the ossicular system in concur-_e with a reduction of the tympanic membrane sticity Unfortunately, there are only two cases cholesteatoma and generalizations must be pered by the smallness of the sample, but results of these 2 patients were reliable to *sistent across both cases*

or future studies, it would be of interest to ´lyse whether a relationship exists between • shifting of the W shape towards high fre-encies, and the degree of stapes fixation ilarly, it would be useful to study a large up of post stapedectomized ears, using tym-ogram configuration in order to determine effect of the different surgical techniques and thesis generally used (area of the exposed l window, mass and length of the prosthesis, bility of its attachment to the long process he incus, and so on) Studies in progress -cate that pre- and post operative compara- tympanometric examinations might well w the evolution of a post-stapedectomized to be followed on an individual basis

RESUME

modifications du tympanogramme dans l intervalle requence de 200 a 2 000 Hz sont ete etudiées Le panogramme, en augmentant la frequence d explora en continuité, presente de caracteristiques modifica de sa forme, qui sont constamment observables l oreille normale aussi que dans les differentes mala-de l oreille moyenne Une atipie de ce comportement lier est representee par celles conditions patologiques lesquelles la dynamique au bien la structure du me tympano-ossiculier est sensiblement alteree tervalle de frequence à laquelle les différentes tym-grammes sont incluses representent, dans notre experience, l element capable de suggerer des informations diagnostiques dans les differentes conditions physiopatologiques de l oreille moyenne

ZUSAMMENFASSUNG

Im Frequenzbereich von 200 bis 2 000 Hz sind die Änderungen der Tympanogramme von Normalhorenden und Patienten mit unterschiedlichen Mittelohrkrankheiten untersucht worden Bei wachsender Frequenz des Testtons zeigt das Tympanogramm sowohl bei Normalhorenden als auch bei einer Reihe von Mittelohrerkrankungen durchgehend bestimmte charakteristische Veranderungen Die einzige Ausnahme in diesem regelmassigen Schema sind die Falle, sei denen im pathologischen Bild die Struktur oder die Dynamik des Tympano-ossikular-Systems deutlich anomal ist namlich bei Cholesteatomen und bei Otitis media, hier weist das Tympanogramm keine Änderung gegenuber der Frequenz auf Aus den Untersuchungen lasst sich entnehmen dass der Frequenzbereich der eine der charakteristischen Formen des Tympanogramms erkennen lasst, als solcher ein diagnostisches Indizium fur die Differenzierung physiopathologischer Veranderungen im Mittelohr ist

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V. Colletti, M.D.
2nd ENT Dept.
University of Padua
Verona
Italy

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ᴧODIFICATIONS OF THE STAPEDIUS MUSCLE REFLEX UNDER SPONTANEOUS AND EXPERIMENTAL BRAIN-STEM IMPAIRMENT

A. Bosatra, M. Russolo and P Polı

From the Department of Otolarʒngologʒ, Unıversıty of Trıeste, Trıeste, Italy

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ract An oscılloscopıc plus a graphıc method of rdıng of the stapedıus muscle reflex has revealed lıar modıfıcatıons ın the latency, threshold, and e of development of the reflex ın cases of braın-stem ırment. Many subjects have been examıned who affected by nucleo-retıcular vestıbular syndrome, ular ınsuffıcıency, dıssemınated sclerosıs, tumours, and a comparıson has also been made ın normal ·cts and ın the same subjects durıng a temporary ırment of the braın stem structures followıng ıturate admınıstratıon The greatest semeıologıcal ꜫ ıs attrıbuted to the modıfıcatıons ın the shape of eflex

as already been observed that the relatıve-edance studıes of the mıddle-ear muscle xes, orıgınally destıned to ınvestıgate the ›ıo pathologıcal condıtıons of the mıddle-ꜫffector structures, have subsequently proved ncreasıng value also ın studyıng the func-al condıtıons of theır supportıng nervous ı Thus, a lowerıng of the physıologıcal shold gap of the stapedıus reflex has been onstrated ın cases of end-organ deafness, ı e he begınnıng of the afferent arch, thus rıng an objectıve demonstratıon of the uıtment phenomenon (Metz, 1951, Lıden, ı)

ıbsequently, an abnormally rapıd decay of reflex contractıon, due to an ımpaırment of VIII nerve fıbres, has been demonstrated, thıs can be a very precocıous sıgn of acoustıc oma (Anderson et al , 1970)

the same way, an ınterruptıon of the ent arch of the stapedıus reflex reveals ›ıte of damage along the VII nerve

Very few reports have so far appeared regardıng *modıfıcatıons of the stapedıus reflex* due to damage of the ıntermedıate portıon of the nervous arch, between the cochlear and the facıal nucleı

In a prevıous note (Bosatra & Russolo, 1970) we have descrıbed the cupula-lıke pattern of the stapedıus reflex as traced by a graphıc recorder ın patıents affected by a moderate, temporary ımpaırment of the braın-stem structures (acute nucleo-retıcular vestıbular syndrome) due to exogenous or endogenous ıntoxıcatıon In these cases the hearıng at threshold and suprathresh-old levels was normal and the lesıon was characterızed by spontaneous and strumental sıgns of vestıbular ımpaırment

In these same cases also the latency of the reflex and ıts threshold were ıncreased (Bosatra & Russolo, 1973, Bosatra et al , 1973)

In thıs paper we wıll refer to the modıfıca-tıons of the varıous parameters of the stapedıus reflex as observed ın varıous types of patho-logıcal condıtıons and durıng experımental temporary ımpaırment of the functıon of the central nervous system, wıth predomınant effect at the level of the braın-stem structures, obtaıned by barbıturate admınıstratıon

MATERIAL

The followıng subjects have been examıned

(1) 25 healthy ındıvıduals of normal weıght and body constıtutıon, aged 15–35, wıth normal

hearing (10–15 dB SPL) and normal tympanometry

(2) The same 25 individuals, during the temporary impairment of the tonic activity of the central nervous system, especially evident at the brain stem level, obtained by barbiturate administration (sodium pentobarbital 3–4 mg/kg, per os)

(3) 15 patients affected by acute nucleoreticular vestibular syndrome, showing normal hearing and tympanometric tracings but complaining of vertigo, with spontaneous nystagmus of varying intensity and direction, hyporeflectivity and saccadic E N G tracings

(4) 10 patients effected by cerebral vascular insufficiency, with clinical, E N G and E E G signs of preminent lesion at brain stem level, with slight presbyacusis (hearing threshold from 10 to 50 dB SPL, with normal tympanometry) and complaining of attacks of vertigo and showing spontaneous nystagmus of varying intensity

(5) 15 patients affected by disseminated sclerosis with normal hearing (10–15 dB SPL) but with spontaneous nystagmus, hyperreflectivity, with irregular E N G tracings and other neurological signs of the disease

(6) 2 patients affected by brain stem tumour (astrocytoma) Both patients showed slight deafness with symmetrical hearing threshold at 30–40 dB SPL with spontaneous nystagmus and E N G tracings of central type

(7) 1 patient affected by unilateral, extrameatal ponto-cerebellar angle tumour, with well preserved hearing (30 dB SPL), bilateral spontaneous nystagmus and homolateral brainstem involvement

(8) 1 patient affected by syringobulbia

METHODS

The stapedius muscle reflex was studied by using a Madsen ZO 70 or Madsen ZO 72 Impedance Meter connected to a graphic recorder and to a dual beam storage oscilloscope

The subjects were examined in a silent room, sitting or lying on a stretcher, with closed eyes, avoiding head movements The reflex was elicited by pure tones of 4 000-2 000-10… Hz presented through an earphone in the … scribed order

The reflex threshold was detected throu… graphic recorder by using stimuli of 1… duration, 10 μsec rise and fall time, at in… steps of 1 dB

The reflex latency was measured at 1… over-threshold and at 120 dB SPL, by us… dual beam storage oscilloscope The … of the reflex was evaluated by averag… responses elicited by tones of 500 msec du… 10 μsec rise and fall time, identical for fr… and intensity The stimulus repetition wa… dom, every 25–30 sec The successive oscill… tracings of the reflex were memorized … first channel and the series of the tonal … was memorized and superimposed on the … channel

The reflex shape has been graphically … at 10 dB overthreshold with the same … tones, of 10 sec duration The onset … reflex therefore has been examined both … the oscilloscope and the graphic recorder

APPARATUS

A function generator Unaohm EM 95 A … used for pure tones, the duration, the ris… fall time, the intensity of the test tones … controlled by an electronic switch (G… Stadler 1287), an attenuator at 1 dB … (Grason-Stadler 1292), an amplifier (G…

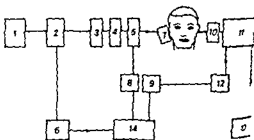
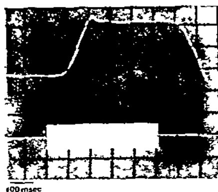
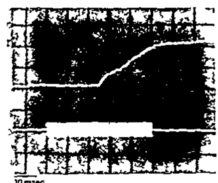


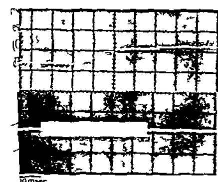
Fig 1 Instrumentation block diagram 1 F… generator 2, Electronic switch 3 1 dB steps at… 4 Amplifier 5 5 dB steps attenuator 6 T… Earphone 8 9 Differential amplifiers 10, … 11 Impedance meter 12 Preamplifier 13 … paper recorder 14 Oscilloscope



2 Oscilloscope tracing in normal subject Not latency and sharp rising of the reflex *A* reflex stimulus (1 000 Hz)



3 Oscilloscope tracing in the same subject of Fig ng barbiturate action Note longer latency and slow like rise *A* reflex *B* stimulus (1 000 Hz)



4 Oscilloscope tracing of stapedius reflex in acute o-reticular vestibular lesion Note longer latency slow step-like rise *A* reflex *B* stimulus (1 000 Hz)

ller 1288) an attenuator at 5 dB steps ason Stadler 1293)

he order of tone presentation and the trigger of the oscilloscope were controlled simultaneously by a series of timers (Grason Stadler 1216 A)

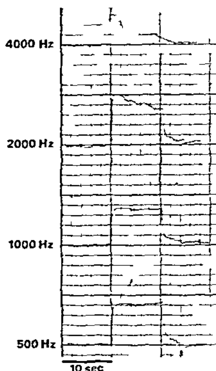


Fig 5 Graphic record of stapedius reflex in normal subject

The tones were presented through an earphone (Fujiki DR 59) The responses were studied by a Madsen ZO 70 or ZO 72 Impedance Meter connected to a graphic recorder Goerz Minigor RE 501 and to a dual beam storage oscilloscope Tektronix 5103 N/D 13 (Fig 1)

RESULTS

Reflex threshold

In the normal subjects the threshold at the four frequencies (4 000–2 000–1 000–500 Hz) ranged from 80 to 90 dB SPL Under pathological conditions the values altered as follows

Subjects under the action of the barbiturate 90 to 95 dB SPL

Patients affected by acute nucleo-reticular vestibular syndrome (n r v s) 90 to 95 dB SPL

Patients affected by cerebral vascular insufficiency *80 to 120 dB SPL*

Patients affected by disseminated sclerosis 80

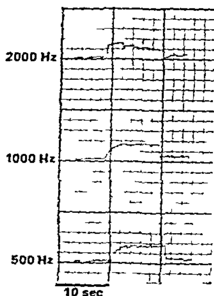


Fig 6 Graphic record of stapedius reflex in same normal subject of Fig. 5 during barbiturate action Note cupula like pattern during the rising phase of the reflex and reduced amplitude

120 dB SPL In 4 subjects belonging to this group examined during an acute phase of the disease the reflex was absent In 3 out of the same 4 patients tested after some months during a phase of clinical recovery the reflex threshold was obtained within normal values at 1 000 and 500 Hz and at 110 dB SPL at 4 000 and 2 000 Hz

Patients affected by brain stem tumour bilateral absence of the reflex

Patient affected by unilateral ponto-cerebellar angle tumour reflex present at 110 dB SPL for 1 000 and 500 Hz only with strong decay homolateral to the lesion (where the earphone was applied) Reflex present at 100 dB SPL but peculiar cupula like pattern on the contro-lateral side

Patient affected by syringobulbia reflex bilaterally absent

Latency

In normal subjects the latency of the st m r examined with the above mentioned methods ranged from 40 to 180 msec

Under pathological conditions the latency was always lengthened as follows

Subjects under barbiturate action fro[illegible] 390 msec

Patients affected by acute n r v s fro[illegible] 360 msec

Patients affected by brain stem impa[illegible] to disseminated sclerosis or vascul[illegible] ciency and also patient affected by [illegible] ponto-cerebellar angle tumour fro[illegible] 400 msec

Shape of the reflex tracings

The formal aspect of the st m r el[illegible] dB overthreshold has been examined [illegible] oscilloscope and a graphic recorder (see M[illegible]

In normal subjects the graphic or [illegible] scopic tracing was rising at the onse[illegible] sharply (Figs 2 5)

In the same subject during the barb[illegible] effect (Fig 3) and in several of the pa[illegible] affected by brain stem impairment, d[illegible] nucleo-reticular lesion (Fig 4) disse[illegible]

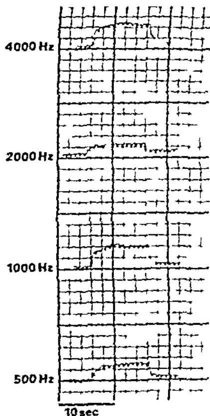


Fig 7 Graphic record in acute nucleo-reticular lesion Note slow onset of the reflex

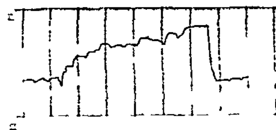


Fig 8 Enlarged graphic record of stapedius reflex in nucleo-reticular vestibular lesion 1 000 Hz, 10 dB over threshold 10 sec duration

	A		B		C	
uencies	St m r threshold	Latency at 120 dB	St m r threshold	Latency at 120 dB	St m r threshold	Latency at 120 dB
0 Hz	100 dB	190 msec	95 dB	190 msec	90 dB	170 msec
0 Hz	100 dB	170 msec	95 dB	160 msec	90 dB	140 msec
0 Hz	85 dB	160 msec	85 dB	150 msec	85 dB	120 msec
0 Hz	95 dB	160 msec	95 dB	150 msec	95 dB	120 msec

9 Modifications of various parameters of stapedius cle reflex during disseminated sclerosis (A) Acute phase (B) During recovery (after 10 days) (C) Complete recovery (after 3 months)

sclerosis, etc, the oscilloscopic tracing was rising slowly with peculiar saccadic steps

With the graphic recorder the same phenomenon appeared as a cupula like pattern of the rising phase

This peculiar shape of the tracings was regularly present in the barbiturate group (Fig 6), quite regularly present in the patients affected by acute n r v s (Figs 7, 8), less regularly present in the cases of vascular insufficiency or disseminated sclerosis

In these same cases the amplitude of the responses was often reduced, but in the vascular patients it was sometime increased However, there was no tendency towards a fast decay

It can be added that in all pathological conditions the tracings appeared to be less stable and regular than in the normal ones

CONCLUSION

By applying an oscilloscopic plus a graphic method of recording of the stapedius reflex we have observed peculiar modifications in cases of pathological or experimental impairment of the central nervous system and especially at the level of the brain stem

Thus the modifications of the reflex can be attributed to an impairment of the central portion of its nervous reflex arch This concerns the threshold the latency and the shape and development of the reflex contraction especially during the rising phase This latter phenomenon is the more easily appreciable and therefore we believe it bears the greatest semeiological significance

It can be suggested that this peculiar shape represents a sort of desynchronization of the conduction of the stimulus at the brain stem nucleo-reticular level

In some cases of vascular insufficiency, disseminated sclerosis, tumours and syringobulbia, we have observed an absence of the reflex This has been already described in a few other cases by Greisen & Rasmussen (1970)

The absence may also be of se[illegible] value, especially when a bilateral co[illegible] can be made, or during the course of th[illegible] as we have observed in some of [illegible] disseminated sclerosis (see Fig 9)

ZUSAMMENFASSUNG

Durch die Anwendung einer Methode mit Osz[illegible] und graphischen Geraten zur Reflexregistr[illegible] M stapedius konnten bei einer Reizung des [illegible] encephalicus Veranderungen der Latenz, der [illegible] und der Reflexformen registriert werden. Es [illegible] Patienten untersucht worden die vestibulare, [illegible] retikuläre Syndrome aufwiesen sowie vaskul[illegible] zienz, Sklerose Tumoren usw Weiterhin ist [illegible] gleich zwischen Patienten gezogen worden, b[illegible] zuerst keine Behandlung mit Barb turaten und [illegible] eine derartige Behandlung durchgeführt wur[illegible] an die trunco-enzephal schen Strukturen zu [illegible] Der grösste Wert des Experiments besteht in d[illegible] nung der Veranderung der Art des Reflexes

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A Bosatra M D
Dept of Otolaryngology
University of Trieste
Trieste
Italy

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SISI TEST AND ADAPTATION

II *Subjects with Conductive Hearing Defects*

T. Rahko

From the Department of Otolaryngology, University of Oulu, Oulu, Finland

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The effect of adaptation of the ear on the SISI was studied at 500 Hz and 2 000 Hz in 43 patients chronic otitis and in 20 patients with otosclerosis mean adaptation was slightly over 10 dB, except in otosclerosis group at 2 000 Hz, for which the was nearly 20 dB after continuous stimulation at B SL for 3 min The mean SISI values ranged from to 31 % in the preadaptation tests and from 9 % % in the postadaptation tests The scatter of the pre postadaptation SISI scores was wide, making tical treatment of the results difficult In the total rial 22 % of the values at 2 000 Hz were positive rding to Jerger's classification, and 32 % were tionable Adaptation slightly reduced the proportion ositive values, but there were changes towards both and lower levels in the cases of chronic otitis, all the otosclerosis changes were diminutions

effect of adaptation on the SISI test in 26 ects with normal hearing has been reported er (Rahko, 1971) After the conventional had been administered, the test ear was pted for 3 min with a sustained 20 dB ation level tone SISI scores of between and 6 000 Hz were obtained immediately rwards The statistical dispersion of both pre- and postadaptation values was found to large Adaptation caused no significant age in either direction in the SISI scores, me of which increased and some decreased out 15 % fell within the positive group, according to the classification of Jerger (1961) itially, Jerger (1959) found that the SISI es in his material of 21 subjects with contive hearing defects, of 5 to 60 dB at 1 000 and of 15 to 60 dB at 4 000 Hz, ranged from 0 % to 15 % at both frequencies No diagnostic classification of the patients was given In a later study (*1961*), he reported negative *SISI* scores in 15 cases, questionable ones in 4 and positive ones in 2 The SISI scores were now classified as follows 0–15 % negative, 20–55 % questionable and >60 % positive The recruitment phenomenon, measured using Fowler's alternate binaural loudness balance (ABLB) was negative in all cases Similar low SISI scores were also obtained by Young & Harbert (1967) in cases of conductive hearing defects

The suprathreshold adaptation tests (Palva 1955, 1964, Kärja, 1968, Palva & Karjä, 1969) have shown that even normal ears develop a marked adaptation at 20 dB sensation level (SL) No tests have been performed on ears with conductive hearing defects at this level At 60 dB level and 2 000 Hz Karjä (1970) found adaptation to be at its maximum, of the order of 30 dB, in cases of otosclerosis and of chronic otitis media

In the SISI test the 20 dB SL tone lasts 1 min 40 sec, which permits considerable adaptation

The present study was carried out in order to investigate the SISI values in conductive hearing defects, and to test whether adaptation affects these values

MATERIAL AND METHODS

The series consisted of 63 outpatients, 38 women and 25 men, mean age 32 years, at the Oulu University Department of Otolaryngology

study was aided with a grant from the State Medical arch Council

Table I *Average audiological findings of the conductive material in various groups*

Frequency (Hz)	Pure tone hearing thresholds (dB, ISO) — Air conduction — AE Mean	S D	CE Mean	S D	Bone conduction — AE Mean	S D	CE Mean	S D	3 min tone decay test (dB) — AE Mean	S D	CE Mean	S D	Recruitment AE when 100 dB SL: Number of ... amount of ... — None	partial 10-25 dB	(-
Chronic otitis															
500	41 1	10 0	8 0	8 5	8 0	8 6			3 2	4 0	2 7	2 6	33	10	0
2 000	34 7	10 7	6 5	5 8	5 7	6 4	5 0	6 1	8 0	8 4	5 8	6 3	29	14	0
Otosclerosis															
500	43 0	10 7	9 5	6 3	10 8	6 5	10 0	3 5	5 0	3 0	2.0	2 6	12	8	0
2 000	36 8	11 4	8 0	6 6	11 3	9 2	5 0	3.5	5 8	6 9	5.3	6 0	15	5	0
Total material															
500	41 7	10 2	8 6	7 8	7 9	5 6	10 0	3 2	4 0	3 6	2 4	2 6	45	18	0
2 000	34 8	11 7	6 9	6 1	7 3	7 8	4 5	4 7	7 3	8 0	5 5	6 0	44	19	0

They all had unilateral conductive hearing defects, with normal hearing in the contralateral ear 44 had chronic otitis media and 20 had otosclerosis

Only those patients for whom the difference between the hearing thresholds did not exceed 50 dB were included in the analysis of suprathreshold adaptation In this situation, with ne normal ear, interaural overhearing does ot affect the examination of suprathreshold adaptation when insert receivers are used (Palva & Palva, 1962) Suprathreshold adaptation was measured at 500 Hz in 35 patients with chronic otitis media, at 2 000 Hz in 41 such patients, and at both frequencies in all 20 cases of otosclerosis

The following basic audiological tests were performed on every patient A pure tone audiogram was taken using the ascending-descending method with air and bone conduction thresholds The equipment used was a Madsen OB 60 audiometer with TDH 39 earphones calibrated according to the ISO standard The bone conduction thresholds of the control ear were not always ascertained, provided that the air conduction threshold was normal Speech audiometry was carried out using the Finnish language test of (1952), measuring the speech reception thr (SRT) and also the speech discrimination at a level of 30 dB above the SRT The e ment used for this test comprised a M SU 20 speech unit connected with a M OB 60 audiometer

A 3 min threshold adaptation test w cluded by increasing the adapting tone in steps in the Madsen OB 60 audiometer test was not performed on all patients a Hz, unless the 2 000 Hz value had ind adaptation Loudness recruitment was mea according to the Fowler ABLB techniq suitable cases filtered speech test after (1965) was also performed However, d large threshold differences, the filtratio could be carried out on only 7 patients 5 chronic otitis and 2 with otosclerosis

Further details of the equipment and me were given in an earlier paper (Rahko, 19 modification on the earlier technique wa use of insert ear receivers for the re given above The equipment was calibra eral times during the test period

The test began with the audiological eva tion described above The SISI test was

e II *Average hearing thresholds with Bekesy audiometer (re 0 0002 dyn/cm²) and balance levels re 3 min 20 dB suprathreshold adaptation (SL) in control ear*

	Chronic otitis						Otosclerosis						Total material					
	Hearing thresholds				Balance levels		Hearing thresholds				Balance levels		Hearing thresholds				Balance levels	
	Adapt ear		Control ear		Control ear		Adapt ear		Control ear		Control ear		Adapt ear		Control ear		Control ear	
	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D
Hz	51 8	15 0	14 4	10 8	32.8	12 5	50 3	13 2	17 2	11 7	34 9	12 6	51 3	14 3	15 3	11 0	33 5	12.5
Hz	33 3	12.6	4 3	8 2	23 9	12.3	31 7	10 7	1 7	8 4	32 7	14 9	33 0	12.0	3 7	8 2	26 7	13 7

inistered on the conductively impaired ear 0 dB SL using 1 dB intensity pulses After ort pause the patient balanced the 20 dB pulsed tone in the test ear with a similar ed tone in the control ear The tone in the ear was then made continuous for 3 min, ‹e the subject balanced it with a pulsed in the control ear and recorded the result SISI increments were then added im piately to the adapted continuous tone in ear to be examined The test frequencies 500 Hz and 2 000 Hz, and the masking employed using 50–70 dB white noise ective level) in the control ear (Palva & za, 1962)

the purposes of the statistical evaluation threshold adaptation was measured 15, 45 60 90, 120, 150 and 180 sec after the of the test The reference value l was the preadaptation balancing level of control ear to the 20 dB SL tone given to test ear The results were processed at the University Computing Centre After deter ation of mean values and standard devia s the differences between the averaged ilts were examined using the Student s test differences were considered significant when 0 01

RESULTS

audiological findings from the basic tests given in Table I The air conduction thres ds for the two groups do not vary much, and though the 3 min threshold adaptation test showed slightly less adaptation in the control ear in both groups, the difference was not significant

The recruitment phenomenon was incomplete in less than one-quarter of the cases with chronic otitis at 500 Hz and in one third at 2 000 Hz. Incomplete recruitment occurred in two fifths of the cases of otosclerosis at 500 Hz and in a quarter at 2 000 Hz Complete loudness balance was never observed at the 100 dB maximum level (SL) for the control ear, the partial recruitment ranging from 10 dB to 25 dB

In the total series the SRT was 40 4 dB in the test ear and 9 4 dB in the control ear, while for the chronic otitis patients the figures were 40 4 dB and 8 8 dB, and for those with oto-sclerosis 41 3 dB and 10 8 dB, respectively The average speech discrimination score for the total material was 96 8 % for the test ear and 98 7 % for the control ear, the intra group differences being of the order of 1 % The average discrimination score in the filtered speech test was 82 9 % for the test ear, 86 3 % for the control ear and 81 9 % binaurally The sub-groups are too small to warrant comparison

In the group analysed for suprathreshold adaptation the difference between the air conduction thresholds of the test ear and control ear averaged between 33 3 dB and 28 4 dB, depending on the frequency and group

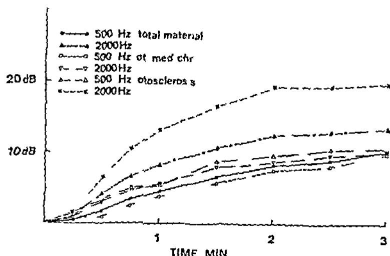


Fig 1 *Perstimulatory suprathresh*[illegible] tation as a function of frequency [illegible] min testing with sustained tone [illegible] SL.

Table II shows the mean thresholds measured with a Bekesy audiometer re 0 0002 dyn/cm² and the balancing levels in the control ear (SL) prior to adaptation, classified according to the type of defect and the frequency used The lowest balancing level was recorded in the group with chronic otitis at 2 000 Hz. All the balancing levels exceeded 20 dB, the values being higher at 500 Hz than at 2 000 Hz

Fig 1 shows the adaptation as a function of time Maximum adaptation was recorded at 2 000 Hz in the otosclerosis patients Adaptation seemed to occur within the first two minutes, after which time changes were small

The mean SISI scores for the various test groups before and after 20 dB suprathreshold adaptation for 3 min are presented in Table III The mean preadaptation values ranged from 15 0 % to 31 1 %, and the postadaptation values from 9 0 % to 30 7 % On average there seemed to be a slight lowering in the SISI score, but due to the wide scatter found in the results, these differences were not significant Figs. 2 [illegible] show the individual results in the [illegible] groups before and after adaptation

Analysis of the SISI values for each [illegible] subject according to the Jerger classi[illegible] (1961) shows that the negative and questi[illegible] groups were the largest before and after [illegible] tion (Table IV) However, the proporti[illegible] positive values in the chronic otitis grou[illegible] 26 % at 2 000 Hz, and 22 % in the total [illegible] Positive values were more numerous ge[illegible] at 2 000 Hz than at 500 Hz, though the p[illegible] tion of positive results among the posta[illegible] tion SISI values seemed to be slightly [illegible] than elsewhere

A transition from one Jerger grou[illegible] another occurred in six of the 43 pa[illegible] with chronic otitis at 500 Hz and in [illegible] 2 000 Hz. The changes were evenly distr[illegible] in both directions In the otosclerosis [illegible] the figures were 2 and 4 out of a total of [illegible] all the changes were in the diminishing dir[illegible]

Table III *Average SISI scores before and after adaptation in different groups of the coductive* [illegible]

Frequency	Chronic otitis				Otosclerosis				Total material			
	Pre adapt		Post adapt		Pre adapt		Post adapt		Pre adapt		Post [illegible]	
	Mean %	S D	Mean %	S D	Mean %	S D	Mean %	S D	Mean %	S D	Mean %	SD
500 Hz	21 4	19 2	20 1	24 0	15 8	22 0	9 0	16 8	19 6	20 1	16 8	[illegible]
2 000 Hz	31 1	28 2	30 7	28 6	22.0	20 9	20 5	22.2	28 2	26 3	27 4	[illegible]

36% of the chronic otitis cases at 500 Hz 76% at 2 000 Hz no change was shown corresponding ratios for the otosclerosis s were 90% and 80%, and for the total erial 87% and 78% No transition from tive to negative or vice versa was observed ould thus be said that the majority of the values did not change their Jerger group r a 20 dB suprathreshold adaptation of 3

DISCUSSION

mal SISI scores in cases of conductive cts were defined in the original article of er (1959) as lying in the range 0–15% er (1961), he reported 15 instances of a ative SISI score, 4 of a questionable score 2 of a positive score, in a material of 21 es with conductive defects The average adaptation values in the range 15–31% ained in the present work are higher than se reported by Jerger, and a comparison of ults for the total series here with those of ger (1961) shows that 22% of all cases in the sent series gave positive values at 2 000 Hz, against approx 10% in Jerger's material wever, Jerger did not specify the frequency In the chronic otitis group the per- tage of positive *SISI* values was higher, %, at 2 000 Hz, which differs even more Jerger's values Questionable values at

ble IV *SISU scores grouped using Jerger's fication before and after 3 min 20 dB SL ptation*

quency)	Before adaptation 0–15%	20–55%	60–100%	After adaptation 0–15%	20–55%	60–100%
ronic otitis						
500	21	18	3	22	17	3
2 000	19	12	11	16	17	9
osclerosis						
500	14	5	1	15	5	0
2 000	10	7	3	13	5	2
tal material						
500	35	23	4	37	22	3
2 000	29	20	14	29	22	11

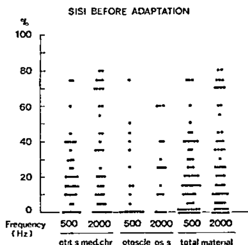


Fig 2 Individual SISI scores at various frequencies in the pre adaptation test

2 000 Hz occurred in 32% of cases, as against approx. 24% found by Jerger (1961) In both subgroups in the present series the 2 000 Hz results differ from Jerger's values more than do the 500 Hz results

Student's *t* test comparison of the present SISI values for conductive deafness with those found earlier (Rahko, 1971) in subjects with normal hearing revealed no differences between the preadaptation groups ($p > 0.01$), but a significant difference at 500 Hz in the post adaptation groups between the otosclerosis patients and the normal subjects ($p < 0.01$)

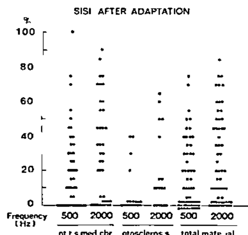


Fig 3 Individual SISI scores at various frequencies in the post-adaptation test

A very wide range of SISI values was found in the present study Therefore, as pointed out earlier (Rahko, 1971), the results support the recommendations of Yantis & Decker (1964) that the limit for positive results should be raised to 80 %, and of Hanley & Utting (1965) that an increment smaller than 1 dB should be used On the other hand, partial recruitment, which has not as a rule been considered to be a feature of conductive deafness, occurred in 30 % of the material at 2 000 Hz and in 29 % at 500 Hz. There were no cases of mixed deafness, however, and the recruitment apparently is mechanical in nature (Anderson & Barr, 1966, Palva et al, 1974)

Suprathreshold adaptation has not been studied earlier at the 20 dB SL in connection with conductive hearing defects, adaptation with 60 dB SL 2 000 Hz tone exceeded that at lower frequencies (Kärjä, 1970) The otosclerosis group in the present series is more in keeping with this trend, whereas the suprathreshold adaptation of the chronic otitis group was practically the same at both frequencies A statistical comparison with the normal material (1971) revealed no significant differences in suprathreshold adaptation in any of the groups

The effect of adaptation on the SISI test in cases of conductive deafness seems to be negligible The mean values for the total material showed a slightly declining trend, but the change was not statistically significant The difference was greatest in the otosclerosis group at 500 Hz, a decline from 15 % to 9 %, while other changes were considerably smaller Individual transitions from one Jerger group to another were more frequent in both directions in the chronic otitis than in the otosclerosis group, where the trend was predominantly a declining one There was not a single change from positive (60–100 %) to negative (0–15 %) or vice versa 87 % of the SISI scores at 500 Hz and 78 % at 2 000 Hz did not change from one Jerger's group to another

Subsequent to suprathreshold adaptation, the subjective tone level in the SISI test had fallen by more than 10 dB, and in otosclerosis patients at 2 000 Hz by even more. [illegible] this adaptation had no statistically [illegible] influence on the SISI test This [illegible] Jerger's statement (1959) that a cont[illegible] in the SISI test does not affect its res[illegible] statement can now be extended to [illegible] to cases of conductive hearing defe[illegible] subject of any of the test groups repor[illegible] disappearance of the continuous adapt[illegible] This concurs with the nature of the de[illegible] which no major adaptation is to be ex[illegible]

A similar study is being conducted on p[illegible] tive hearing defects

ZUSAMMENFASSUNG

Die Wirkung der Adaptation des Ohres auf de[illegible] Test wurde an 43 Patienten mit chronischer [illegible] ohrentzundung und an 20 Patienten mit Otoskle[illegible] 500 und 2 000 Hz untersucht Die durchsch[illegible] Adaptation betrug etwas über 10 dB Nur d[illegible] sklerosegruppe wies bei 2 000 Hz und nach [illegible] kontinuierlicher Stimulation einen Wert von an[illegible] 20 dB auf Beim Praadaptationstest varie[illegible] durchschnittlichen SISI Werte zwischen 15 0 un[illegible] und postadaptatorisch zwischen 9 0 und 30 7 [illegible] Streuung der praadaptatorischen SISI Werte w[illegible] und erschwerte die statistische Auswertung N[illegible] Klassifikation von Jerger gab es bei 2 000 Hz 2[illegible] tive Werte im gesamtem Material und 32 % frag[illegible] Werte auf derselben Frequenz Die Adaptati[illegible] ringerte die positiven Werten etwas, und zwa[illegible] nehmender Richtung Nur in der Otosklero[illegible] waren samtliche Abweichungen in abnehmend[illegible] tung

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T Rahko, M D
Dept of Otolaryngology
University of Oulu
SF 901 00 Oulu
Finland

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SISI TEST AND ADAPTATION

III Subjects with Perceptive Hearing Defects

T Rahko

From the Department of Otolaryngology, University of Oulu Oulu, Finland

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Abstract The effect of the adaptation of the ear on the SISI test was studied in 60 completely and 18 incompletely recruiting patients and in 5 patients with a verified acoustic neurinoma at 500 and 2 000 Hz. The subjective suprathreshold adaptation to a 3 min tone of 20 dB (SL) ranged from 10 dB to over 20 dB depending on the frequency and type of defect The pre adaptation mean SISI values in the recruiting groups ranged from 38 to 51 % and the post adaptation means from 38 to 49 %. In retrocochlear lesions only one SISI value was question able before and after adaptation, the others being 0 %. The dispersion of the SISI scores was considerable, a fact

be borne in mind

In his original article on the SISI test, Jerger et al (1959) reported also on the values he had obtained in perceptive deafness In nine cases of Menière s disease the scores were from 70 to 100 % and 95 to 100 % respectively, in 34 cases of presbyacusis the scores varied from 0 to 100 % and in three cases of retrocohlear lesions they were 0 % at 500 and 2 000 Hz

In a later article, Jerger (1961) reported on 20 cases of unilateral Meniere's disease, all with a positive SISI score, on 11 cases of neurinoma of the acoustic nerve including nine with a positive and two with a questionable SISI score, and on perceptive unilateral hearing defects of undefined diagnosis, as follows 16 completely recruiting ears of which 13 had a positive 2 a questionable, and one a negative SISI score, 22 partially recruiting ears of which 15 had a positive, 4 a questionable, and 3 a nega[illegible] score, and 37 non recruiting ears of w[illegible] had a positive, 2 a questionable, and 5 a ne[illegible] SISI score

Yantis & Decker (1964) found that, [illegible] Hz out of 18 years with a retrocochlear [illegible] 16 had a SISI score of 0 % and the rem[illegible] two 60 and 95 %, whereas of 42 ears [illegible] cochlear lesion 22 had a SISI score of [illegible] and most of the remaining 20 had a SISI exceeding 80 %. At 2 000 Hz, in the material, the SISI score was 0 % in 13 ret[illegible] lear lesions and 100 % in 32 cochlear lesion[illegible]

Owens (1965) described 95 cochlear lesi[illegible] which 91 had a SISI score exceeding 6[illegible] one of the frequencies used, viz. 500 Hz, [illegible] Hz, 2 000 Hz or 4 000 Hz In 12 ears retrocochlear lesions the SISI score was [illegible] In 7 patients the host tone faded away [illegible] a few seconds, in 3 the tone faded away b[illegible] patients heard 2-3 dB increments, wher[illegible] two the tone did not fade away at all an[illegible] patients heard the 2-3 dB increments but [illegible] smaller ones

Hughes (1968) selected for his st[illegible] patients in whom the SISI host tone [illegible] away but the SISI scores exceeded 75 % [illegible] at two of the frequencies studied Fifteen [illegible] patients had either a vascular or [illegible] based cochlear lesion and three had an [illegible] neurinoma, later verified surgically

Young & Harbert (1967) described [illegible] lateral, recruiting sensorineural flat type [illegible]

cts in which the SISI scores exceeded 60% ı 20 dB sensation level, and 13 cases with ıological adaptation (over 20 dB) in which ırdless of the performance level of the test, SISI scores were with two exceptions 0-5% y stated that in conductive and sensorineural ring defects, excepting those with pathological ptation, the SISI test was positive if the nd pressure level (SPL) entering the inner exceeded 60 dB

uprathreshold adaptation in perceptive hear defects has not been studied at a sensation l of 20 dB, although Palva (1952) reported suprathreshold adaptation was at its ıest at 45 dB in recruiting hearing defects in non recruiting ears at 20 dB

he duration of the SISI test is 1 min 40 sec therefore, as is known from the supra-shold adaptation studies by Palva (1952 and 4), Kärjä (1968) and Palva & Kärja (1969), siderable adaptation of the host tone must place The present study investigated the ad of the SISI scores in perceptive hearing ects of different types, the effect of adaptation these scores and compared them with the a obtained earlier in normals and in con-tion deafness (Rahko, 1971 and 1974)

MATERIAL AND METHODS

material consisted of 83 subjects, 37 men and 46 men, mean age 43 years All had unilateral perceptive hearing defect Five ıents had an acoustic neurinoma verified gically, or in one case, by contrast medium iography None of these ears showed louds recruitment The completely recruiting up of 60 patients contained 32 with Menière's ease and 7 with a definitely vascular cochlear on, the remainder having a lesion of unde-ed etiology The incompletely recruiting group 18 patients contained two with Meniere's ease and 4 with a vascular cochlear lesion, remainder having a perceptive loss of un ıned etiology Recruitment was considered nplete when with 100 dB (SL) the difference in loudness levels between the ears was ±5 dB A recruitment deficit of 10 dB in the test ear, or more, at 100 dB (SL) was defined as incomplete recruitment

Only those patients in whom the differences between hearing thresholds did not exceed 50 dB were included in the analysis of suprathreshold adaptation The effect of overhearing was minimized using the insert receivers in the investigation of suprathreshold adaptation (Palva & Palva, 1962)

The basic audiological examination of the patients was the same as described earlier (Rahko, 1974) They were subjected to pure tone and speech audiometry (Palva, 1952), to a 3-minute threshold adaptation test, recruitment test according to Fowler (ABLB) and hearing thresholds and interaural differences permitting to a filtered speech test (Palva, 1965)

The SISI test procedure was similar to that described earlier (Rahko, 1974) The SISI test was given to the ear with the perceptive hearing defect at 20 dB sensation level (SL) using 1 dB pulses After a short interval the patient balanced the 20 dB (SL) pulsed tone given to his affected ear with a similar pulsed tone in the control ear The tone to the affected ear was then given continuously for 3 min and the test subject balanced it with a pulsating tone in the control ear making a recording with self-recording audiometer Immediately after this the SISI increments were added to the continuous adapted tone in the tested ear The test frequencies were 500 and 2 000 Hz During the SISI test masking in the control ear consisted of 50–70 dB white noise (Palva & Palva, 1962)

For statistical treatment, the suprathreshold adaptation was measured at points 15, 30, 45, 60, 90, 120, 150 and 180 sec The reference level used was the pre-adaptation level of balancing the tone in the control ear with the 20 dB (SL) tone given to the tested ear The results were computerized at the Oulu University Computer Centre After the mean values and standard deviations had been determined the extent of the differences between the mean results was studied using the Student's *t* test The differences were

Table I *Average audiological findings of the perceptive deafness material*

Type and frequency (Hz)	Pure tone thresholds (dB ISO)				3 min tone decay test (dB)				Recruitment, … 100 dB SL in … Number of s…	
	Ae		Ce		Ae		Ce			
	Mean	S D	Mean	S D	Mean	S D	Mean	S D	None	Inc…
Incomplete recruitment										
500	40 8	19 6	10 0	4 7	7 0	4 8	5 0	5.3	—	18
2 000	51 7	20 7	12 2	7 5	27 3	22 8	15 2	22 0	—	19
Complete recruitment										
500	47 6	17 9	11 9	7 4	8 2	9 7	3 4	4 9	—	—
2 000	44 0	16 3	10 8	9 6	21 1	25 5	16 6	25 1	—	—
Retrocochlear										
500	46 0	23 8	11 0	7 4	18 0	19 6	6 3	9 5	5	—
2 000	67 5	6 5	15 0	12.2	28 8	13 8	5 0	7 0	5	—

Ae = adapting test ear, Ce = control ear

classified as significant if p was smaller than or equal to 0 01

RESULTS

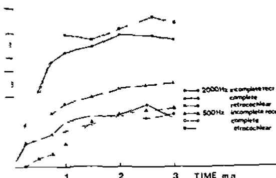
The results of the basic audiological tests are seen in Table I The pure tone thresholds of the groups differed little, apart from the retrocochlear group at 2 000 Hz In the 3-min threshold adaptation test, the tested ear in all groups showed a slightly higher degree of adaptation than the control ear The difference was greatest the retrocochlear group Recruitment was lete, depending on the frequency, in 59–60 cases and incomplete in 18–19

The patients with incomplete recruitment at 2 000 Hz had a mean speech reception threshold (SRT) of 46 0 dB in the tested ear an in the control ear, and in the group plete recruitment the mean SRT for ear was 44 2 dB and for the control ea *The corresponding mean speech d* tion scores were 64 7 and 96 4 %, and 96 9 %, respectively *In the retr* group the SRT was 48 3 dB in the and 13 8 dB in the control ear, and crimination scores 38 3 and 100 0 %, res The differences between the recruitin were thus fairly small

The filtered speech test could be carri *only nine patients The mean results,* Hz were grouped as follows the te control ear and binaural results, in

Table II *Average hearing thresholds with Békésy audiometer (re 0 0002 dyn/cm²) and bala before 3 min 20 dB (SL) suprathreshold adaptation in control ear Perceptive material*

Frequency (Hz)	Incomplete recruitment						Complete recruitment						Retrocochlear			
	Hearing thresholds (dB)				Balance levels (dB)		Hearing thresholds (dB)				Balance levels (dB)		Hearing thresholds (dB)			
	Test ear		Control ear		Control ear		Test ear		Control ear		Control ear		Test ear		Control ear	
	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D
500	50 2	22 9	17 4	12 1	33 3	12 0	56 4	16 4	19 6	11 7	35 9	17 8	41 0	11 5	8 4	8 7
2 000	47 2	17 7	9 4	8 4	35 4	16 2	39 4	16 5	7 5	10 3	33 0	15 3	61 3	11 7	7 0	4 4



1 Perstimulatory suprathreshold adaptation as a ion of frequency and type of hearing defect during ı testing with sustained tones at 20 dB (SL)

plete recruitment group were 63 5, 80 0 68 0 %, respectively, and in the complete uitment group 76 6, 67 0 and 66 8 %. The lts were too few for statistical treatment

able II shows the mean values for pure tone shold measured with the Bekesy audiometer 0002 dyn/cm² and the balance levels in the rol ear before adaptation, grouped accord to the hearing defect type and frequency

ig 1 shows the adaptation as a function ime Maximum adaptation was obtained at 0 Hz in cases of retrocochlear hearing defect ost the full degree of adaptation seems to lop within the first 2 min Adaptation at 0 Hz seems to exceed that at 500 Hz in tically all groups At 500 Hz it is of the r of 10 dB

he mean SISI values before and after a 3 min athreshold adaptation at 20 dB are presented able III by test groups The pre adaptation ies ranged from 38 0 % to 50 9 % in the re cruiting groups and from 4 6 % to 11 3 % in the retrocochlear defect group After adaptation the figures were 38 3 % to 49 4 % and 7 1 % to 12 5 % respectively The mean values of all groups increase, but no statistical significance is obtained owing to the great dispersion

Figs 2 and 3 give the individual results graphically As can be seen, the rectrocochlear defect group contains only one value differing from 0 %

The analysis of the SISI values according to the Jerger classification (1961) presented in Table IV shows that the positive and negative values were most numerous in the recruiting groups, whereas the retrocochlear defect group contained only one questionable value, the others being negative In the incomplete recruitment group, at 2 000 Hz, the positive and negative SISI values before and after adaptation were equal in number whereas at 500 Hz there were fewer negative than positive values In the completely recruiting group the situation concerning the frequencies was the reverse In the retrocochlear defect group there were no changes, and the majority of the SISI values were negative Individually, transitions from one Jerger group to another are few, and 95–79 % remained un changed depending upon the type of defect and the frequency No transition from positive to negative or vice versa was found

DISCUSSION

In his material of 17 patients with acoustic nerve trauma or Meniere s disease Jerger (1959) reported SISI values of 70–100 % in cochlear

le III *Average SISI scores before and after adaptation in different groups of perceptive material*

	Incomplete recruitment				Complete recruitment				Retrocochlear			
	Pre adapt		Post adapt		Pre-adapt		Post-adapt		Pre adapt.		Post adapt	
uency	Mean (%)	S D	Mean (%)	S D	Mean (%)	S D	Mean (%)	S D	Mean (%)	S D	Mean (%)	S D
)	46 4	38 1	49 4	39 4	38 0	33 2	41 6	35 0	4 6	10.3	7 0	15 7
)	42.1	36 5	38 3	37 7	50 9	38 6	49 4	39 6	11 3	22.5	12.5	25 0

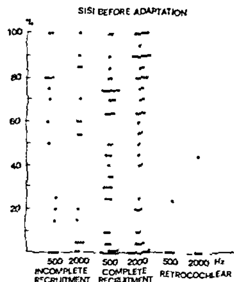


Fig 2 Individual SISI scores at various frequencies and in different types of hearing defects in the pre adaptation test

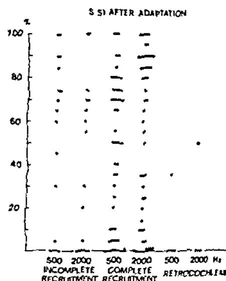


Fig 3 Individual SISI scores at various freque *in different types of hearing defects in the post-a* test.

defects In a later article (1961) *Jerger described* 20 cases of Meniere's disease Five of these patients had no recruitment phenomenon, and *the SISI scores were positive in all cases* In the same study, Jerger listed 11 acoustic neurinomas, the SISI test being negative in 10 and questionable in one The SISI values obtained for the *present retrocochlear defect group, viz. four* negative and one questionable, agree with *Jerger's results On the other hand, the mean* scores obtained in the recruiting groups in- recruitment 46 4 and 42 1 %, and com- recruitment 38 0 and 50 9 %, are distinctly lower than Jerger's values The SISI test was positive, according to the Jerger classification, *in the incomplete recruitment group* of cases at 2 000 Hz, questional in 1 negative in 41 %, and similarly positive questionable *in 16 % and negative in* 500 Hz. In the completely recruiting g *percentages were 53, 17 and 30 at 2* and 38, 26 and 36 % at 500 Hz Henc *recruiting groups the SISI was positive* most, in 53 and at least in 38 % of the

The results of the present study agr those reported by Yantis & Decker as far as retrocochlear hearing defects a cerned, but differ concerning the cochlear for which they reported 83 % positiv values, 5 % negative and the remainer q

Table IV *SISI scores grouped using Jerger's classification before and after 3 min 20 dB (S. rathreshold adaptation*

Freq (Hz)	Before adaptation			After adaptation		
	0–15 %	20–55 %	60–100 %	0–15 %	20–55 %	60 100 %
Incomplete recruitment						
500	6	3	9	6	2	10
2 000	7	3	7	7	3	7
Complete recruitment						
500	20	15	21	20	13	23
2 000	17	10	31	20	9	29
Retrocochlear						
500	4	1	—	4	1	—
2 000	3	1	—	3	1	—

at 500 Hz, and 92 % positive and 2 % nega- at 2 000 Hz Similarly, the present results r in the group of cochlear recruiting lesions those reported by Owens (1965*a*, *b*) In of these two studies the SISI test failed to ositive in only 5 out of 95 cochlear lesions ast in one of the frequencies used, and in other study the SISI was positive in 70 out recruiting perceptive lesions, i e 97 %

a comparison with those of earlier studies ko, 1971 and 1974), using the Student's , the pre-adaptation SISI values of the olete recruitment group at 2 000 Hz are ficantly bigger than those of otosclerosis rial and very significantly bigger than ial material At 500 Hz, the mean pre- tation SISI values of the two groups of itment in the present material are very ficantly bigger than those of the otosclerosis p and significantly bigger than those of nic otitis group In post-adaptation SISI the complete and incomplete recruitment p SISI values of this study also were highly ficantly bigger at 500 Hz as compared with onductive defect groups, but at 2 000 Hz in the complete recruitment group of this SISI values were significantly bigger than of the otosclerosis group

e SISI values for the present group had a large dispersion Positive values were ded for 53–38 % in the recruiting groups at ent frequencies On the other hand, in all one of the retrocochlear lesions (acoustic nomas) the SISI test was 0 % Hence, on the of the present and earlier results (Rahko, and 1974), it may be concluded that a SISI value does not reliably make a nosis regarding the locus of pathology in ng defects Neither does a negative SISI listinguish different kinds of hearing defects oly from normal and from each other ko 1971 and 1974), but then the possibility retrocochlear lesion must always be care- ruled out

prathreshold adaptation in perceptive de- has not previously been studied at the B sensation level In the present material, adaptation in retrocochlear lesions was at its maximum at 2 000 Hz, the host tone fading away in two cases It was next greatest in completely recruiting hearing defects and small- est in incomplete recruitment At 500 Hz the adaptation in all types of defects was of the 10 dB order The decay of the host tone also agrees with Owens' results (1965) in seven of the 11 cases of a acoustic neurinoma the host tone faded away A comparison of the degree of adaptation with the normal and conductive defect materials (Rahko, 1971 and 1974) revealed no statistically significant differences between the groups

Comparisons of the present mean SISI values in the various groups before and after adaptation reveal no consistent trend of change in either direction The changes themselves are numeri- cally small, and no statistically significant differences could be shown between the groups The number of acoustic neurinomas is naturally too small for a reliable comparison A study of the results per individual reveals no transition from a positive to a negative Jerger group or vice versa In the incomplete recruitment group at 500 and 2 000 Hz 79 % remained unchanged, as did 85 % in the complete recruitment group at 500 Hz and 95 % at 2 000 Hz The changes that occurred were fully symmetrical in both directions, except at 2 000 Hz in the completely recruiting defect group where all changes were diminutions

In the SISI test following a suprathreshold adaptation of 3 min at 20 dB (SL), the sub- jective intensity of the host tone had diminished by 10 to over 20 dB depending on the type of de- fect, and had completely faded away in two cases, but this did not seem to affect the SISI test signifi- cantly This agrees with Jerger's (1959) claim that changes in loudness of the sustained host tone do not affect the result of the SISI test this has in the present study been demonstrated to apply also to perceptive hearing defects

ZUSAMMENFASSUNG

Die Wirkung der Adaptation im Ohr auf den SISI Test wurde bei 60 vollkommen recruitierenden und 18 un-

vollkommen recrutierenden Personen sowie bei 5 zuverlassig verifierten Retrocochlearläsionen (Akusticus neurinom) bei 500 Hz und 2 000 Hz untersucht Die subjektive Grosse der Adaptation uber der Schwelle bei 3 Min 20 dB (SL) varierte je nach der Frequenz und dem Fehlertypus von 10 dB bis uber 20 dB In den recruttie renden Gruppen ersteckten sich die durchschnittlichen Werte bei dem Praadaptations SISI Test von 38 % bis 51 % und bei dem Post-adaptations SISI Test von 38 % bis 49 %. Bei den Retrocochlearläsionen war der SISI Wert nur in einem Fall pra und postadaptatorisch fragwurdig sonst 0 %. Die Dispersion der SISI Test Werte war recht gross was die statistische Behandlung des Materials schwierig machte Die Adaptation hatte keinen besonderen Einfluss auf den SISI Test Wert Es konnte festgestellt werden dass hohe SISI Test Werte nicht zuverlassig Horfehlertypen voneinander unter scheiden. Wenn der SISI Test Wert aber niedrig ist muss die Moglichkeit der Retrocochleärlasion immer beachtet werden

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T Rahko M D
Dept of Otolaryngology
University of Oulu
SF 90100 Oulu 10
Finland

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THE USE OF THE DRILL IN EAR SURGERY

O Hallen and A Tjellström

From the Department of Otolaryngology, University of Goteborg, Goteborg, Sweden

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act The surgeon of today has at his disposal a large l of technical aids For the ear surgeon it is pri the rotating drill which has helped him in the ting theatre In spite of the fact that the drill is an important tool in daily operative procedures roblems concerning drills have been accorded singly little attention in the medical press It is noticeable when visiting various clinics in different of the world that there appears to be little correla etween surgical standards obtained and the quality e technical equipment used The object of this is to illustrate some of those problems which exist rning the use of the drill in oto-surgery

ing—a question of cutting

ther one works with an ordinary cutting a finishing burr, or a diamond burr, the ing principle is the same Fig 1 illustrates sequence of events in front of the single The cutting edge and its positioning against material which is to be cut can be charac ed by three different angles The bevel e is the angle which is made between the ng edge s facet surfaces The clearance angle ie angle between the lower facet and the rial which is to be cut Finally, the rake e is the angle between the upper facet and plane which is at right angles to the material e cut The optimal positioning of the rake le bevel angle, and clearance angle, are known when used in industry for working both metals of different degrees of hardness well as upon wood However, the ideal ditions for cutting bone have not been rmined On the other hand, such an investi on is hardly necessary The precision which equired in industrial work is mainly a result of the economics of massproduction Considerations as such are thus irrelevant as far as ear surgery is considered, where the limits for acceptable working conditions are relatively wide and rather different Heat production must be minimised in order to prevent traumatising the surrounding bone tissue This means that the rake angle must be relatively large and the bevel angle relatively small The most common cause of excess heat production is without doubt the use of dull burrs The edges become dull either by heavy use or by corrosion The situation can be avoided either by using cheap carbon steel drills (which rust easily) and throwing them away after each operation, or by using stainless steel drills or hard metal drills (corrosion resistant) for repeated use Even with correct cutting speed, correct pressure, and a fine quality cutting edge, it is impossible to avoid con siderable heat production unless the process is cooled by saline solution

Another factor of importance is mechanically induced trauma A cutting burr provided with high edges allows the surface to be worked faster, which is obviously an advantage in certain



Fig 1 The sequence of cutting work

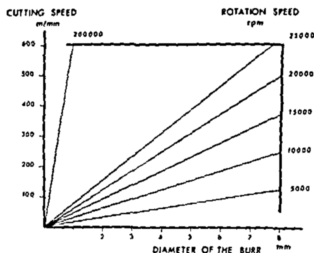


Fig 2 The relationship between the burr's diameter the rotation speed and the cutting speed

stages of ear surgery Such burrs, however, considerably increase the risk of mechanical traumatisation of remaining bone as well as non-mineralized tissue The otologist has long been aware of these risks In more intricate phases of the operation he therefore uses a cutter with a shallow bevelled edge, such as a finishing burr In this respect, diamond burrs *of different stone size are a useful alternative*

Cutting speed—rotation speed range

Yet another important parameter in mechanical tting work is the cutting speed, defined as the locity with which the single edge is moved against the material to be worked With a rotating burr it is the rotation speed and the diameter of the burr which determine the cutting speed This relationship is illustrated in Fig 2

As far as dentistry is concerned two different areas of rotation speed have developed High-speed rotors working between 200 000 and 400 000 rpm on the one hand, and on the other hand low-speed machines working with speeds up to 300 000 rpm

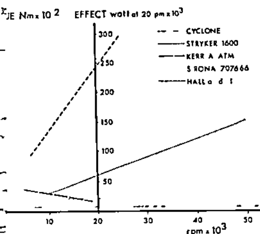
For the ear surgeon there is no question of choosing between these two different areas of rotation speed because of the relationship between the rotation speed and the larg rr diameter which the rotation speed al f th tion speed is exceeded, there

of "throw off", i e that pieces of the edge break away and are thrown away drill In ear surgery one must be a with a burr diameter up to 8 mm in the first stages of mastoidectomy terms this means that if one intends same drill during the different stag operation, one must choose the low spe

There are still no published inv which define the optimal cutting spe working with bone Fig 2 illustrates restricted to the low speed area (at 2000 a burr with a diameter of 8 mm has a speed of around 500 m per minute wh with a diameter of only 1 mm has a c speed of 50 m/min In order to obtain th cutting speed with the smaller burr d it would be necessary to increase the r speed from 20 000 to something over rpm Since the rotation speed of the r has a maximum of 20 000 rpm the r that under certain stages of an operat must accept a cutting speed which is However, since this is not a question of in production, the reduced working speed *results from a lower cutting speed can be* to combine rather well with the high pre stages of an ear operation in which burr smaller diameter are used

Effect

The most common way of relating the pow a drill is to describe its torque, which is days described in Nm (Neutonmeter) torque produced is the tangential power the rotor produces multiplied by the distance the movement's axle, i e the length of the A different way of giving the power of a which is now used, is to define the so braking effect which is thus expressed in or horsepower This gives a more readily stood figure describing the drill's ab work against a resistance The torque, as the braking effect, varies with the r speed of the drill In the majority of cases works at the maximum speed and it is th effect around 20 000 rpm which is most inte



The braking effect at different rotation speeds for of the drills available

hen working in ear surgery, the required ng effect varies considerably during dif- t phases of an operation When, for ple, boring out a cell system with a thick al bone, one uses a large diameter burr a powerful pressure in order to obtain the st working speed possible In this stage demands on the braking effect of the rotor very high On the other hand, when doing work and using a small burr diameter, requirements in this respect are very small

Fig 3 the braking effect and torque of a ber of drills at present on the market are ibed An important difference between ric- and air-driven rotors is that the electric- n rotor with increased demands on torque mes hot, which is a disadvantage if the r is in direct contact with the handpiece thus in contact with the surgeon's hand

handpiece

eneral, two types of handpiece are used in surgery—a straight handpiece or an angled dpiece with a 20–30° angle The function of handpiece is to relay the movement from motor to the head of the drill Two factors involved, firstly, the burr must be securely ored to the handpiece to avoid slipping pushing forward Secondly, the movement t be carefully centred in order to give high working precision It is widely accepted that the handpiece should be no wider than a pen in order to avoid obscuring the view of the working area This means that one usually uses a handpiece designed for dental use However, a characteristic of ear surgery is that in certain stages of an operation a large diameter burr is used under pressure in order to give the fastest possible working speed This results in a considerable radial stress on the handpiece The dimensions of a narrow handpiece result in a poor construction which is not designed for heavy, radially directed pressure This means that the bearings of the drill receive an unusual amount of wear and tear, with consequent reduction of precision i e, increase in the radial throw-off and heating up of the handpiece Such handpieces will obviously wear out quickly

Specifications required of a drill for use in ear surgery

There are a large number of drills to choose from on today's market Apart from considerations of rotating speed and effectivity, several other factors should be appraised when making a choice *The size* of the drill is determined principally by the construction of the drill itself Today most of the older types of drill with belt transmission and rotating cable respectively have been replaced by smaller, electric- or air driven rotors which are directly connected to the handpiece of the drill In these machines, both size and weight are designed to be practicable to work with

Another question of importance *is the ease of sterilising* the drill Naturally it is an advantage if the drill as a complete unit can be autoclaved The risk of contamination is reduced —especially important when changing handpieces However, this obviously sets high demands on the specifications of both material and construction of the rotor, which reflects itself in the price of the equipment Sometimes while operating, the burr catches in soft tissue whereby damage easily follows From this point of view it would be an advantage if the drill can be stopped abruptly Providing that the

NAME Type	SPEED rpm × 10^3	BRAKING EFFECT at 20 × 10^3 rpm watt	OPERATING MEDIUM	OPERATING PRESSURE $Nm^2 \times 10^4$	FLOW liters/min	AUTOCLAVABLE
Cyclone standard	0 - 22	250	air	45	200	no
Cyclone-Kirurg W Weck air drill	0 - 25	250	air	50	170	yes
Hall air drill	0 -100	8	nitrogen	70	180	yes
Stryker 1600	0 - 50	60	air	60	57	yes
Sirona 707666	4 - 40	23	electricity	-	-	no
Kerr A-ATM	0 - 20	6	electricity	-	-	no

Fig 4 Some properties of different drills The Cyclone Kirurg W rotor is identical with Weck air drill

qualities described above are satisfactory, the deciding factors in the choice of a drill are reliability, durability and price Quality in terms of reliability and durability by far outweigh a higher price Fig 4 illustrates data of drills obtainable at the present time

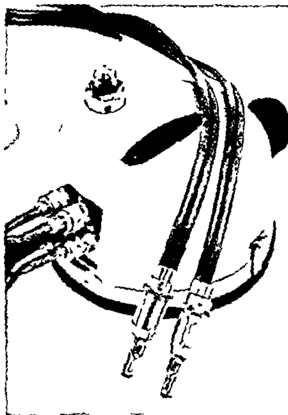
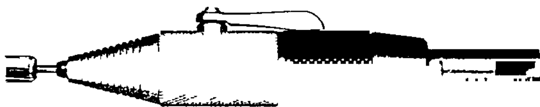


Fig 5 The unit consisting of a footplate and two rotors

Our solution

An objective comparison between differe[illegible] of handpiece and drill has never been m[illegible] would be difficult to carry out Workin[illegible] tions, economics, personal taste and specia[illegible] vary considerably between different su[illegible] It would therefore be wrong to try to gi[illegible] thing but general requirements when cho[illegible] handpiece and drill for ear surgery

However, we would finally like to [illegible] for our choice, based on the conditions at p[illegible] existing in our clinic For the last 10 ye[illegible] have worked with the Cyclone rotor (sta[illegible] The range of rotation speed is 0–20 000 r[illegible] the braking effect highly satisfactory (F[illegible] The rotor cannot be sterilized and a steril[illegible] is thus used On the other hand the [illegible] cheap and has shown itself to be highly r[illegible] By working together with the manufactu[illegible] have developed a unit which consists of a[illegible] plate to which two rotors can be quickly a[illegible] (Fig 5) One of the rotors has a stra[illegible] other an angled handpiece In this way t[illegible] always a handpiece at hand which s[illegible] needs arising during any particular sta[illegible]



The straight handpiece equipped with strong bear nd chuck

ition The complete unit with two rotors less than half the price of a sterilisable Three units cover our needs adequately oximately 500 operations/year) Thanks to ow cost of the unit, both the research labs he residents' bone lab have been equipped Cyclone rotors

hile working with the manufacturer we also developed a special, straight handpiece 6) This handpiece is autoclavable, has strong bearings which can stand heavy, lly applied pressure, and is also supplied an especially strong chuck, which allows changes of burrs and grips firmly enough at the end of the burr axle This is of im ance, in this way the head of the drill be extended, the length depending on the tion during operation In the first stage of a oidectomy, when a burr of wide diameter sed, and when a fast working speed is ired and the radial stress is at a maximum, ort a burr as possible is used, i e the burr naximally displaced into the handpiece later stage of the operation—such as work he antrum atticus region—burrs of smaller are used and the radial stress is small At this e the burr can be displaced forward so that the chuck grips the end of the burr axle This means that instead of using a narrow handpiece, the drill head s axle is lengthened, which allows easy inspection and accessibility The handpiece is cheap and proven to be of excellent durability Only in certain stages of ear surgery—such as transmeatal work—has it proved necessary to use the angled handpiece

ZUSAMMENFASSUNG

Der Chirurg von heute verfugt uber eine grosse Auswahl technischer Hilfsmittel Dem Otochirurgen hilft in erster Linie die Dentalbohrmaschine bei seinen operativen Einsatzen Eine Bohrmaschine bei der selten Betriebs storungen auftreten und die nicht warmlauft schenkt dem Chirurgen Zufriedenheit eine schlecht funktionierende Maschine ist jedoch ein dauernder Grund zur Irritation Obwohl die Bohrmaschine einen wichtigen Bestandteil bei taglichen operativen Einsatzen darstellt ist dem Problem Bohrmaschine in der Fachliteratur bemerkens wert wenig Beachtung geschenkt worden Es ist ebenso bermerkenswert dass man wenn man verschiedene Kliniken in der Welt besucht keine hohere Korrelation zwischen dem chirurgischen Standard und dem Niveau der benutzten technischen Ausrustung findet Dieser Artikel mochte die existierenden Probleme behandeln, die Bohrmaschine in der Otochirurgi behefteu

O Hallen M D
Dept of Otolaryngology
University of Goteborg
S-413 45 Goteborg
Sweden

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COCHLEAR DAMAGE RESULTING FROM KANAMYCIN AND FUROSEMI[illegible]

R E Brummett, J Traynor, R Brown and Donna Himes

From the Kresge Hearing Research Laboratory, University of Oregon Medical School, Portland, Ore, [illegible]

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Abstract Permanent cochlear damage has been shown to occur in guinea pigs following the combined administration of kanamycin and furosemide At the doses used, only a transient effect was measured with furosemide alone and no effect was detectable with kanamycin alone This interaction results when a single subcutaneous dose of 400 mg/kg of kanamycin is followed in 2 hours by a single intravenous dose of furosemide The dosage range for furosemide was 50 mg/kg for a just-detectable effect to 100 mg/kg for a very severe effect Damage to the cochlea was ascertained by measures of the a c. cochlear potential as well as surface preparation histology

The aminoglycoside antibiotic kanamycin can interact with the diuretic ethacrynic acid to produce a severe permanent hearing loss This interaction has been reported in patients (Mathog & Klein, 1969, Johnson & Hamilton, 1970) and in addition has been verified in experimental animals (West et al, 1973) The onset of deafness occurs shortly after the administration of the ethacrynic acid and if a sufficiently large dose of both drugs has been given the deafness is permanent This permanent deafness is the result of hair cell destruction which has been demonstrated histologically in humans (Matz & Beal, 1969) and in experimental animals (West et al, 1973)

Furosemide is a potent diuretic drug related to ethacrynic acid It has a different chemical structure, but its mechanism of action on the kidney is similar to that of ethacrynic acid Also like ethacrynic acid, furosemide by itself, has been reported to produce temporary hearing losses in man (Schwartz et al, 1970) as well as decreased auditory function in exper[illegible] animals (Mathog et al, 1970, Brown [illegible] Elwee, 1972) Unlike the case with e[illegible] acid there are no clinical cases rep[illegible] indicate that furosemide can inter[illegible] kanamycin to produce permanent d[illegible] Nonetheless, it was thought that such [illegible] action might be possible providing s[illegible] furosemide was given The present inve[illegible] was designed to determine whether or [illegible] interaction between kanamycin and fur[illegible] would produce permanent cochlear dys[illegible] in experimental animals

METHODS

This experiment has been divided into tw[illegible] The first part demonstrates that an inte[illegible] between furosemide and kanamycin does and the second part demonstrates t[illegible] effect is permanent and related to the d[illegible] furosemide

In both parts of this experiment, th[illegible] of the drugs on the ear was monit[illegible] observing the cochlea's ability to gen[illegible] alternating current (a c) cochlear pot[illegible] response to pure tone sound stimuli ([illegible] & Lawrence, 1954) The electrical si[illegible] the cochlea was amplified differentially [illegible] times and recorded using a General [illegible] Model 1900 wave analyzer The sound [illegible] were generated with a Western Ele[illegible] speaker The sound intensity was m[illegible] each frequency for each animal with a [illegible]

This research was supported in part by the grants 2 PO1 NS 09889 and 5 TO1 NS05472.

attached to a ½-inch B & K calibrated ophone (Vernon et al , in press) All sound isities are referred to 1 dyne/cm² Details ie methods employed for producing, and uring the sound stimuli, as well as those to record the a c cochlear potential and ide the possibility of radiation artifact, been described before (Brummett et al ,)

r the first part of the experiment, a total guinea pigs were used They were anesthe- with dial (60 mg/kg) and urethane (240 g) administered interperitoneally A tracheal ula was inserted, through which the animal artificially respirated The rate and depth respiration were so adjusted as to prevent ntaneous contractions of the middle ear cles (Meikle et al , in preparation) Details the methods employed to maintain body perature, expose the round window mem- ne, insert and attach the round window trode and keep the bulla free of accumulated ls have been reported previously (Brummett l, 1972, Mitchell et al , 1973) After the al had been prepared, baseline data on lear function of each animal was obtained he 1 μV sensitivity function of the a c lear potential between 100 Hz and 20 kHz addition, the maximum amount of a c lear potential that the animal could generate kHz and 10 kHz was determined, and it will ferred to as an intensity function Following baseline observations, a single 400 mg/kg of kanamycin was given subcutaneously eriod of 2 hours was allowed to elapse for kanamycin to accumulate in the perilymph he cochlea At the end of this 2-hour period, the 1 μV isopotential sensitivity function maximum a c cochlear potential output kHz and 10 kHz were repeated to ascertain cochlear function had not deteriorated this time, sufficient sound intensity at 1 kHz delivered to the ear so that it would produce ut 150 μV of a c cochlear potential This ount of sound is well below that needed to duce cochlear damage Finally a single 100 /kg dose of furosemide was administered intravenously over a 1-minute injection period through an indwelling cannula in the ipsilateral jugular vein In order to monitor any changes in cochlear function resulting from the administration of these drugs, the a c cochlear potential resulting from the 1 kHz tone was continously followed over a 3 to 4 hour time span

As has been reported (West et al , 1973) for kanamycin and ethacrynic acid, the guinea pigs that had received both the kanamycin and furosemide developed not only a short duration loss of cochlear function, but in addition, a more profound effect occurred 2 hours after the furosemide This second loss in the ability to generate the a c cochlear potential did not appear to return during the time course of this short term experiment

A second study was designed to determine the permanency of this interaction effect A group of 4 guinea pigs were anesthetized with pentobarbital (35 mg/kg) given interperitoneally An indwelling jugular catheter was placed and the animal was prepared for recording a c cochlear potential in a manner so as to prevent postoperative infections (West et al , 1973) Both sensitivity and intensity functions of the a c cochlear potential were determined in one ear After calibrating the sound system, the opening in the bulla was covered with crushed gelfoam and the skin closed with Michel wound clips At this time, a single 400 mg/kg dose of kanamycin was given subcutaneously Two hours later, a single 100 mg/kg dose of furosemide was given intravenously over a 1 minute injection period The indwelling catheter was removed and the skin closed with Michel wound clips The animals were allowed to recover from the anesthesia and were maintained for 30 days in the animal quarters In addition, 4 groups of 4 animals each were treated in an identical fashion except that they received doses of 50, 60 or 70 mg/kg of furosemide in order to establish a dose–response relationship for furosemide with a constant 400 mg/kg dose of kanamycin After the 30 day recovery period, the animals were re anesthetized with dial and urethane and sensitivity and intensity functions of the

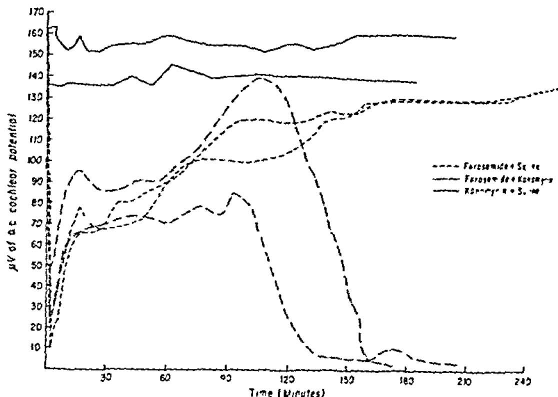


Fig 1 Effects of furosemide (100 mg/kg) and kanamycin (400 mg kg) on the a.c. cochlear potential that is being generated in response to a 1 000 Hz continuous t… kanamycin was given 2 hours prior to the fur…

cochlear potential were determined as described for the short-term experiment However, in this case, both ears were evaluated The temporal bones were saved for histology, the cochlea being perfused with 1 % osmium tetroxide in Dalton's fixative Surface preparation histology was performed to determine the presence or absence of both the inner and outer hair cells (West et al , 1973)

In both the short- and long-term experiments, control animals were run where the kanamycin dose was replaced with saline and the animal then had its normal dose of furosemide Other control animals had their usual dosage of kanamycin, but the furosemide was replaced by saline In either case, the volume of saline was equivalent to that of a proper dose of drug In the case of kanamycin, the drug dosage is calculated as kanamycin base The kanamycin sulfate solution contained 333 mg/ml of kanamycin base For the furosemide, the drug concentration was 10 mg of furosemide per ml

RESULTS

Fig 1 depicts the a c cochlear potentia… obtained at 1 kHz from the short-term e… ment guinea pigs In this instance the a… had received a single 400 mg kg subcut… injection of kanamycin 2 hours prior … 100 mg/kg intravenous injection of furos… It can be seen that the ability of the a… cochlea to generate the a c cochlear p… to the continous 1 kHz tone was rap… pressed after this dose of furosemide… depression was maximum in about 2-4 … whereupon it gradually returned toward c… levels over a 2-hour time period Th… drop in a c cochlear potential output … return to control values is typical of the e… furosemide by itself At this time and … any further drug administration the a c c… potential was again depressed Th… depression was not followed by any rec…

Kanamycin by itself did not produ… effect on the a c cochlear potential w…



› Mean 1 μV sensitivity functions obtained 30 days the guinea pigs had received a single 400 mg kg itaneous dose of kanamycin followed in 2 hours by ing intravenous doses of furosemide The control drug) range from all animals (16) is shown in the led area Mean data from the 8 ears of 4 guinea pigs ich dose of furosemide

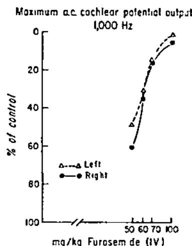
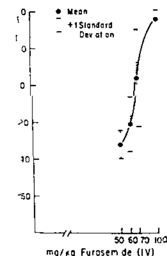


Fig 4 Dose-response relationship of a single dose of kanamycin followed by differing doses of furosemide on the maximum a c cochlear output at 1 000 Hz. Each point represents the average of either 4 right ears or 4 left ears indicated

wed by an intravenous dose of saline larly, furosemide alone only produced the al depression of the a c cochlear potential ı it followed a subcutaneous injection of e It then appears that the second depression inction only occurred when both furosemide kanamycin were given

ɔ determine if this effect on the a c cochlear potential was permanent the long term experiment was performed In this case the animals were injected with kanamycin which was followed in 2 hours with the furosemide In all cases control sensitivity and intensity functions were obtained just before the drugs were given The animals were then surgically closed and allowed to recover for 30 days After this time they were again



3 Dose-response relationship of a single dose of mycin followed by differing doses of furosemide on averaged 1 μV en itivity function Averaged data the 8 ears of 4 guinea pigs for each point

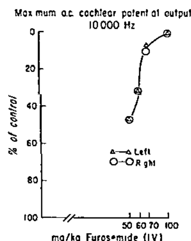
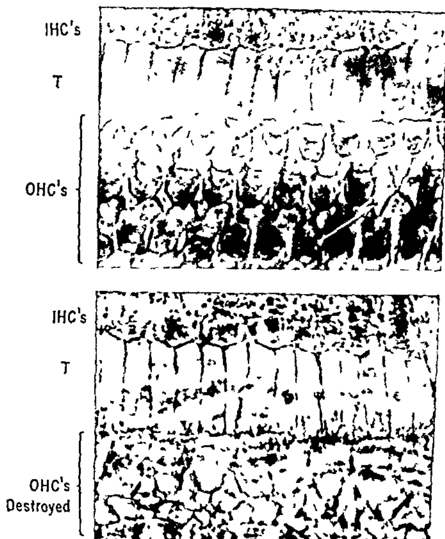


Fig 5 Dose-response relationship of a single dose of kanamycin followed by differing doses of furosemide on the maximum a c cochlear potential output at 10 ... Each point represents the average of either or 4 left ears as

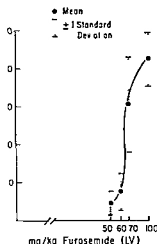


6 Upper A surface preparation from the 2nd turn a guinea pig that had received a single 400 mg/kg of kanamycin followed by a 50 mg/kg dose of furosemide This specimen shows a typical scar from a missing outer hair cell (OHC) that is indicated by the arrow This appears to be a normal specimen *Lower* A surface preparation for the 2nd turn of a guinea pi received a single 400 mg/kg dose of kanamyci by a 70 mg/kg dose of furosemide This speci a total loss of OHC's
(Outer hair cells—OHC s inner hair cell tunnel—T)

evaluated electrophysiologically Fig 2 presents the 1 μv isopotential sensitivity function for these animals The control (pre-drug) data is shown in the stippled area The mean data from the right ears of all animals that had received the different doses of furosemide are shown as single lines As can be seen, the 50 mg/kg dose produced no effect The 60 mg/kg dose produced a possible effect in the high frequencies whereas the 70 and 100 mg/kg dose produced considerable permanent effect when measured 30 days after drug administration

When the sound intensity that is req produce 1 μV of a c cochlear potenti frequencies tested is averaged, one f obtained that we will call the "average sensitivity function" The averaged 1 μ tivity function obtained from the gro guinea pigs that received the same 400 dose of kanamycin but different doses o semide are shown in Fig 3 These data indicate that as the dose of furosemide in the effect on cochlear function increases

In addition to the effect on the 1 μV sens



⁷ Dose-response relationship of a single dose of nycin followed by differing doses of furosemide on ıg outer hair cells Averaged data from the 8 ears of ıea pigs for each point

ıon of the a c cochlear potential, the mum output of the cochlea was determined kHz and 10 kHz For these determinations, sound intensity was increased in 5 dB while the a c cochlear potential that was ɡ generated by the cochlea was monitored was continued until a further 5 dB increase ound pressure produced a decrease in the cochlear potential The maximum output from the intensity function determinations plotted in Figs 4 and 5, as a percentage of control values that were obtained before administration At 1 kHz, the mean con- value was 1387 μV and at 10 kHz it was μV The maximum values that were obtained ı both the left and right ears are plotted arately This shows that the effect on the nals ears is about equal bilaterally It is also arent from the data that the effect at 10 was approximately equal to that at 1 That is to say, the dose of furosemide that ıced the output at both frequencies to 50% the maximum was about 50 mg/kg

inally the number of outer hair cells were ermined in an area from each turn of the hlea Both ears from each animals were nted and the percentage of missing hair s were determined by counting the scars in the cuticular plate resulting from hair cell destruction (Fig 6)

It can be seen in Fig 7 that the number of missing hair cells was also related to the dose of furosemide

DISCUSSION

Our data show that an ototoxic interaction can occur when both kanamycin and furosemide are administered to guinea pigs This interaction occurs between 1½ and 2½ hours after a single 100 mg/kg intravenous dose of furosemide that is given two hours after a single 400 mg/kg subcutaneous dose of kanamycin At this time the a c cochlear potential drops to extremely low values over a 45 to 60 minute time period This drop appears to be independent and possibly produced by a different mechanism than that which produces the initial rapid fall in a c cochlear potential that follows an intravenous injection of furosemide This initial rapid depression of a c cochlear function was monitored and there was no observed difference either in magnitude or time course between animals receiving furosemide alone or those receiving both kanamycin and furosemide

This second drop in the production of the a c cochlear potential appears to be permanent Animals that are examined 30 days after the administration of single doses of kanamycin followed by single doses of furosemide exhibit a permanently depressed ability to generate the a c cochlear potential This change was seen in the sensitivity function of the a c cochlear potential as well as in the maximum amount of a c cochlear potential that could be produced at 1 kHz and 10 kHz Furthermore, at this same time period, there are many hair cells missing from the organ of Corti which is also good evidence for a permanent cochlear effect

When the single dose of kanamycin that was given to these animals is held constant and differing doses of furosemide were administered intravenously a dose-related ototoxic effect occurs This dose related effect was indicated by the a c cochlear potential as well as the number of missing hair cells

In all parameters the measured dose effect curve was very steep in that small increments in dosage produced large effects on the cochlea For instance, the 1 μV sensitivity function of the a c cochlear potential indicated little or no change at 50 mg/kg of furosemide, but exhibited a very marked change at 100 mg/kg Likewise the number of missing hair cells was only about 9 % with a 50 mg/kg dose but 85 % were missing with a 100 mg/kg dose This steep dose response course would mean that cochlear damage would go from little or no effect to severe damage with only a doubling of the dose

One interesting observation is to be made when the dose effect curve for the averaged 1 μV sensitivity function is compared to the dose effect curve for the maximum output In the case of the sensitivity function, the 50 mg/kg dose of furosemide produced little or no change At the same dosage, the effect on the maximum output of the a c cochlear potential was to reduce it to about one half of control values It is possible that changes in the maximum output of a c cochlear potential is a more sensitive indicator of cochlear damage than is the 1 μV sensitivity function

This ototoxic interaction in guinea pigs with kanamycin and furosemide appears to be similar if not identical to that which has been reported or kanamycin and ethacrynic acid in guinea pigs In the case of kanamycin and ethacrynic acid there are reports of similar interactions occurring in man when these two drugs are administered together In the case of ethacrynic acid the dose that produced a very severe interaction effect was 40 mg/kg In the case of furosemide a 50 mg/kg dose produced only a slight interaction effect It may be that the lack of clinical reports is due to the fact that doses of furosemide that are large enough to produce the ototoxic interaction are not given clinically

ZUSAMMENFASSUNG

Es wurde gezeigt dass die Kombination von Kanamycin and Furosemid dauernde Schädigung der Cochlea bei Meerschweinchen verursacht Bei den angewandten Dosen bewirkte Furosemid allein nur eine temporare Schädigung, und nur mit Kanamycin wurde l Wirkung beobachtet Die kombinierte Wirkung sich nach einer subkutanen Injektion von 400 von Kanamycin und einer intravenosen Injekt Furosemid zwei Stunden spater Eine Wirkung gerade noch mit 50 mg/kg Furosemid beobachtet wahrend 100 mg/kg eine sehr starke Wirkung sachten Die Schadigung der Cochlea wurde Messung des Cochlea Wechselstrom Potential durch histologische Oberflachen Präparation be

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R E Brummett Ph D
Kresge Hearing Research Laboratory
University of Oregon Medical School
3515 S W Veterans Rd,
Portland Ore 97201, USA

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IMMEDIATE RESTORATION OF BASAL SENSORINEURAL HEARING (MB MENIERE) USING A PRESSURE CHAMBER

O Densert, S Ingelstedt, A Ivarsson and K Pedersen

From the Department of Otolaryngology, University of Lund, Malmo General Hospital, Malmo, Sweden

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act A new technique for the restoration of basal - neural hearing loss in Mb Meniere was described . cases with unilateral basal sensorineural hearing fullness of the ear, and tinnitus were reported In the stage of their disease the patients were treated in a ure chamber where it was possible to increase or ase the air pressure within the range ±110 cm H_2O equilibration of middle ear pressures to surrounding pressures was checked Exposure to underpressure ed in a rise of the hearing thresholds at low frequencies and relief of subjective symptoms When the air ure was increased the hearing thresholds were ed and the sensation of tinnitus and fullness of the was accentuated Changes in air pressure did not the healthy ear or the high frequencies in the ed ear Hearing improvement attained at exposure derpressure seemed to remain at atmospheric pres evel The investigation was performed on the hypo- that a distended membranous labyrinth might a venous congestion of the vestibular aqueduct ng in impaired endolymph absorption in the endo- iatic sac Possible effects of changes in environmen pressure on the inner ear were discussed

generally accepted today that endolympha- ydrops is the basic feature of Mb Meniere theory is supported by histopathological ngs in Mb Meniere (Hallpike & Cairns Lindsay, 1942, Schuknecht, 1968 Gussen,) A distention of the scala media with acement of Reissner's membrane has been rved in many cases In the vestibular part of ar the distention mainly affects the saccule to a lesser degree, the utricle and the semi- lar canals In a patient with a basal sen ieural hearing loss without vertigo, Lind & Schulthess (1958) found a distention ting only the scala media

It was reported by Stahle (1968) that in 22 % of cases of Meniere's disease the onset was without vestibular symptoms Recurrent periods of basal sensorineural hearing loss, fullness of the ear and tinnitus were the clinical symptoms It is not known how often the symptoms are restricted only to the cochlea and do not include vertigo

In Meniere's disease, glycerol has been used for diagnostic purposes It increases the osmotic pressure of the blood, which might induce a transient reduction of the endolymphatic hydrops In 50 % of the cases, improved hearing resulted (Klockhoff & Lindblom, 1967) Meniere's disease is a recurrent disease with fluctuating symptoms of fullness of the ear, vertigo, deafness and tinnitus Thus, the endolymphatic hydrops is not permanent Beside spontaneous changes it has been possible to affect the hydrops by changing the blood osmolarity

Investigations have been performed in order to evaluate the volume displacement of the middle ear mucosa as correlated to changes in the surrounding air pressure (Andreasson et al, 1975) When the surrounding air pressure was increased, the blood vessels of the middle ear mucosa were congested with blood to compensate for the relative underpressure in the middle ear cavity With decreased air pressure it was possible to record a reduced filling of the blood vessels in the mucosa Provided there was a total or partial obstruction somewhere in the mem-

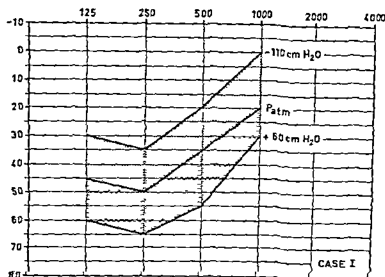


Fig 1 Case 1 Pure tone audiog… shows the hearing level before … exposure to over and unde… Hearing threshold levels were lo… 20 dB at exposure to overpressur… cm H_2O) Short dashes Reduced … sure (−110 cm H_2O) improved th… 15-20 dB Dotted area

branous labyrinth it seemed possible to influence *the volume-pressure relationship Thus, a reduc*tion of the surrounding air pressure might affect the blood volume of veins and capillaries in the inner as well as in the middle ear

Three patients with unilateral acute symptoms of basal sensorineural hearing loss, fullness of the ear, and tinnitus were selected The investigation was performed using a pressure chamber The aim was to record the effect on hearing thresholds from increase and decrease of surrounding air pressure and try to aid the patients by a new therapeutic method

ase histories

Case no 1 B F Female, aged 39 No previous otological disease On 1st October, 1974, symptoms started with tinnitus in her right ear It remained for 3 weeks and was less pronounced at the end of this period On 20th Octo… *tinnitus returned together with fullness of th*… slight vertigo, headache and impaired hea…

Case no 2 G B Female, aged 44 No p… otological disease On 6th November, 19… patient awoke with *fullness of her* righ… tinnitus, and impaired hearing No vertig… morning (Day 2) the symptoms were even pronounced and the patient was admitted ENT-clinic

Case no 3 R H Female, aged 48 Her probably had Meniere's disease On 10th 1974, the patient noticed the first aural… toms fullness of the left ear, impaired h… and an intense sensibility to sounds Sh… admitted to the ENT-clinic where a pur… audiogram revealed a basal sensorineural h… loss on the left ear Within a few d… symptoms disappeared spontaneously, a…

Table 1 *Case 1*

Time (min)	Pressure (cm H_2O)	Fullness	Tinnitus	Vertigo	Pure tone audiogram Hearing loss (dB, ISO 1964) Dx			
					125	250	500	1 000
0	P_{atm}	+ + +	+ + +	0	45	50	35	20
8	+ 60	+ + −	+ + −	0	60	65	55	30
15	− 50	+ −	+ + +	0	40	40	30	10
21	− 90	+	+ −	0	30	30	25	5
26	−110	+	+ +	0	30	35	20	0
33	P_{atm}	+ + +	+ + +	0	45	50	35	15

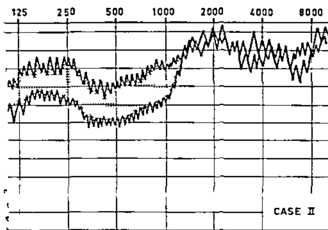


Fig 2 Case 2 Basal sensorineural hearing loss Bekesy audiograms made immediately before and after the exposure to underpressure Dotted area shows the gain in hearing of 15 to 20 dB

July the audiogram was quite normal On December, 1974, the patient awoke with o, fullness, tinnitus, and impaired hearing left ear

METHODS AND EQUIPMENT

logical investigation

treatment in the pressure chamber the of the hearing loss was studied Pure tone grams, acoustic stapedius reflex, acoustic decay, Fowler's test, hearing threshold de st and speech discrimination test were per d Electronystagmography was performed in the pressure chamber audiometer (Madsen, Model OB 70) and ectroacoustic bridge (Madsen, Model ZO calibrated in accordance with ISO n 1964 R389 (International Or- ition for Standardization) and checked to fulfil the requirements of IEC Publication 177 (International Electrotechnical Commission, 1965)

Immediately before and after treatment in the pressure chamber, Bekesy audiograms, with interrupted tones, were performed Conventional tracing where the frequency of the test signal moved gradually upward from 100 to 10 000 cps (Gransen Stadler, Model E-800)

Pressure chamber

A pressure chamber with sound isolation was used It was possible to increase or decrease the air pressure within the range of ± 110 cm H_2O Via a pipe system arranged in special valves the chamber was connected to a high pressure fan by which air could be blown into or evacuated from the chamber (Ingelstedt et al, 1967) The patient was placed in a sitting position inside the chamber Pure tone audiograms were taken at

II *Case 2*

Pressure (cm H_2O)	Fullness	Tinnitus	Vertigo	Pure tone audiogram Hearing loss (dB, ISO 1964) Sin 125	250	500	1 000
P_{atm}	+ + +	+ + +	0	40	40	35	25
30	+ +	+ +	0	30	30	35	15
−50	0	0	0	15	15	25	15
P_{atm}	+ +	+	0	20	25	25	15
P_{atm}	+ +	+	0	30	25	20	15
−50	0	0	0	20	25	15	5
P_{atm}	+	0	0	20	20	15	15

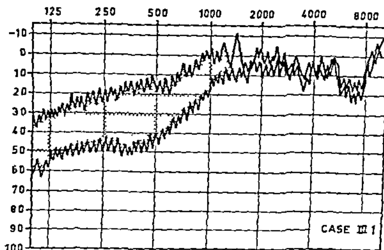


Fig 3 Case 3 Basal sensorineur loss Bekésy audiogram performed and after the stay in the pressure *At the first exposure to* there was a gain in hearing to 25 dB Dotted area

various pressure levels when the middle ear pressure was equilibrated to the pressure level of the chamber The equilibration was checked with an electroacoustic birdge (Madson, Model ZO-72), where the minimum impedance was recorded when the ear drum was at its neutral position All audiograms were performed when the fan was switched off Pure tone audiograms at low frequencies were performed at 125, 250, 500 and 1 000 cps

RESULTS

In all 3 cases, pure tone audiograms showed a basal sensorineural hearing loss in the diseased ear and perfectly normal hearing in the other one The audiological investigation schedule revealed that the sensorineural hearing loss was located in the cochlea

In all 3 cases, electronystagmography performed after treatment in the pressure chamber showed a normal caloric response in bo During the increase or decrease of th pressures, hearing thresholds of the non ear were not affected

Case no 1

The patient was subjected to both increas reduced air pressure in the chamber

Increased chamber pressure *pressure in the chamber was increased cm H_2O (Table I) When the pressure wa* the patient complained of increased ful even pain in the diseased right ear thresholds at low frequencies were depre 20 dB (Fig 1)

Reduced chamber pressure W chamber pressure was reduced, pain and fullness in the diseased ear dim At a pressure level of −90 cm H_2O (hearing thresholds at low frequencies

Table III *Case 3*

Time (min)	Pressure (cm H_2O)	Fullness	Tinnitus	Vertigo	Pure tone audiogram Hearing loss (dB ISO 1964) Sin 125	250	500	1 000
0	P_{atm}	+	+	+	40	45	40	10
7	50	+	+	+	40	45	40	10
49	−50	0	0	0	40	45	40	10
53	−70	0	0	0	40	40	30	5
62	−70	0	0	0	35	35	25	0
121	−70	0	0	0	30	30	20	0
129	P_{atm}	0	0	0	30	25	15	0
154	P_{atm}	0	0	0	30	25	10	0

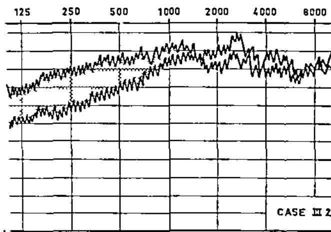


Fig 4 The same patient as in Fig 3 A recurrence occurred 2 days after the first exposure to underpressure Bekesy audiogram The dotted area shows the gain in hearing

10–20 dB compared with the audiogram nospheric pressure (P_{atm}) A further reduc of chamber pressure to −110 cm H_2O l the hearing thresholds slightly (Fig 1) patient had been exposed to reduced air ure for only 13 minutes when it was in ed to P_{atm} At this level the audiogram was stent with the original one at atmospheric ure (P_{atm}) The fullness of the ear had re d

no 2

ie second day (Day 2) after the onset of the patient was treated for one hour pressure chamber at 50 to 80 cm H_2O ctive symptoms and the basal sensorineural ig loss were not affected Two days later 4) the audiogram still had the same con ition (Fig 2) The patient was exposed to pressure and even at −30 cm H_2O the tion of fullness and tinnitus in the diseased anished After 24 minutes at 30 and 50 cm H_2O the gain in hearing thresholds at low frequencies was 10 to 25 dB (Table II) The pres sure in the chamber was raised to atmospheric level (P_{atm}) and the gain in hearing thresholds mostly remained The patient was exposed to −50 cm H_2O once more and the hearing thresh olds were raised again (Table II) The Bekesy audiograms performed before and after expo sure to underpressure are shown in Fig 2 Two days later the audiogram was normal

Case no 3

This patient was exposed to underpressure even on the first day (Day 1) of her disease The treat ment lasted for about 2 hours After about 40 minutes the sensation of vertigo disappeared The rise in hearing thresholds began somewhat later (see Table III) The hearing gain obtained in the chamber remained at P_{atm} and Bekesy audiograms before and after the exposure to underpressure are compared in Fig 3

On day 2 the Bekesy audiogram was practi

IV *Case 3*

Pressure (cm H_2O)	Fullness	Tinnitus	Vertigo	Pure tone audiogram Hearing loss (dB ISO 1964) S n 125	250	500	1 000
P_{atm}	+ +	+	+	30	30	15	0
70	+	0	+	25	25	10	0
−70	0	0	+	25	25	10	0
90	0	0	0	20	15	10	0
90	0	0	0	20	20	10	0
P_{atm}	0	0	0	20	20	10	0

cally normal On the following day (Day 3) all symptoms had returned but the hearing loss was less pronounced After exposure to underpressure of −70 and −90 cm H_2O for one hour (*Table IV*) there was a marked rise in hearing thresholds (Fig 4) One day later (Day 4) the audiogram was normal again

DISCUSSION

Production and reabsorption of the endolymph seem to be the most essential points in the development of endolymphatic hydrops If the endolymphatic sac is of vital importance in the reabsorption of endolymph (Guild, 1927) the endolymphatic duct, from the anatomical point of view, is the place most easily liable to obstruction The vestibular aqueduct is a bony canal which contains the endolymphatic duct In the proximal part, near the vestibulum, it narrows into an isthmus (Ogura & Clemis, 1971) The endolymphatic duct is surrounded by loose connective tissue containing blood vessels The endolymphatic duct and sac probably derive part of their arterial blood supply and venous drainage via the paravestibular aqueduct (Anson, 1965, Ogura & Clemis, 1971)

From experimental studies it is known that endolymph is reabsorbed in the endolymphatic sac (Lundquist, 1965) Kimura & Schuknecht (1965) and Kimura (1967) blocked the endolymphatic duct in guinea pigs Subsequent endolymphatic hydrops was observed In microscopic investigations, abnormalities in the vestibular aqueduct have been observed by Altman & Zechner (1968) who observed a bony occlusion of the duct By means of radiography Clemis & Valvassori (1968) showed a partial or total obliteration of the vestibular aqueduct Further histological studies on temporal bones performed by Ogura & Clemis (1971) and Yuen & Schuknecht (1972) gave contradictory results They did not find any differences in the calibre of the vestibular aqueducts in ears with Meniere s disease as compared with normal ears Most histopathological investigations of Meniere's disease showed a distention of the membranous labyrinth Even in the original desc[illegible] fibrosis of the walls of the endolymph[illegible] was described (Hallpike & Cairns 19[illegible] performing decompression operations [illegible] endolymphatic sac, Shambaugh et al [illegible] observed ischemia of the exposed wall [illegible] many cases partial or complete oblite[illegible] the lumen of the sac Microscopic exam[illegible] showed a reduced vascularity and a mark[illegible] epithelial fibrosis

Thus, there seems to be evidence [illegible] that Meniere's disease is caused by a par[illegible] temporarily total obstruction of the end[illegible]phatic duct Increased intralabyrinthine p[illegible] may result in reduced venous drainag[illegible] venous stasis may affect the flow in the [illegible]lymphatic duct by partial or total obstru[illegible] the duct In previous studies it has been [illegible] that when the environmental air pressur[illegible] raised, blood vessels in the middle ear [illegible] were filled with blood because of the [illegible] underpressure in the middle ear cavity The vessels were emptied when the body was [illegible] to underpressure (Andreasson et al [illegible] There seemed to be many parallels betwe[illegible] middle ear cavity and the inner ear T[illegible] might also be possible to affect veno[illegible] capillary blood filling in the inner ear

Three patients with suspected acute [illegible]lymphatic hydrops are presented and the[illegible] after exposure to increased and decreas[illegible] pressure are described They all had [illegible] basal sensorineural hearing loss with [illegible] and fullness of the affected ear In all c[illegible] was possible to affect hearing threshol[illegible] changes in the environmental air pr[illegible] Exposure to reduced air pressure induced [illegible] in hearing thresholds The improvement [illegible] average, 15–20 dB at a pressure of 50-[illegible] H_2O, and in 2 cases the gain in hearing re[illegible] with the return to atmospheric pressure [illegible] as illustrated by Bekesy audiograms perf[illegible] before and after the spell in the pressure c[illegible]ber In only one case (Case 1) was a p[illegible] pressure applied It resulted in an imm[illegible] decline of hearing thresholds at low frequ[illegible] and the subjective symptoms of fullness

 us were accentuated Symptoms and hear-
resholds again improved with the return
mospheric pressure Exposure to under-
ure resulted in nearly complete relief of
ctive symptoms and improved hearing In
ase the gain in hearing thresholds did not
n, but hearing returned to the initial level
nospheric pressure.

diograms performed in the pressure cham-
ere done when the pressure in the middle
as equilibrated to the level of the pressure
ber The possibility exists that pressure
ges in the inner ear are caused by a volume
acement via the round and oval windows
study on human temporal bones it was
ble to quantify the volume displacement in
ner ear caused by changes in middle ear
ures The recorded volume displacements
small at the pressure of 30–50 cm H_2O
son & Pedersen, 1975) It is therefore con-
ble that changes of the surrounding air
ure will affect the blood volume of the inner
ssels

s not yet possible to quantify the volume
e in the inner ear fluids caused by changes
ous blood congestion Experimental studies
imals, however, are in progress in order to
r this question

idolymphatic duct and sac as well as sen-
epithelial cells The question arises whether
possible to affect all cases with suspected
ymphatic hydrops by changing the sur-
ling air pressure

our experience, cases of Meniere's disease
isorineural hearing loss without vertigo are
oved by exposure to underpressure only
g the acute stage of the disease and re-
nce Sensory cells and membranes damaged
ng standing hydrops will not respond to this
nent

ere are many questions about Meniere's
se which cannot be answered today We
hat the method presented is an improve-
which will make it possible to help the
patient and open up new ways in the investiga-
tion of Meniere's disease

ZUSAMMENFASSUNG

Darstellung einer neuen Methode zur Behandlung von endolymphatischem Hydrops Es werden drei Falle von einseitiger Innenohrschwerhorigkeit mit Horverlust fur tiefe Tone, Druckgefuhl und Ohrgerauschen beschrieben Die Behandlung erfolgte im akuten Krankheitsstadium in

und subjektive Symptomlinderung Drucksteigerungen hingegen resultierten in einer Herabsetzung der Schwellenwerte und Intensivierung der Druckgefuhle und Ohrgeräusche Die Änderungen des Luftdrucks waren ohne

suchung wurde mit der Hypothese durchgefuhrt, dass eine Schwellung des membranosen Labyrinths venose Stockungen im *aqueductus vestibularis* verursachen konnte, die einen verschlechterten Abfluss der Endolymphe im

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O Densert, M D
Dept of Otolaryngology
Malmo General Hospital
S 214 01 Malmö
Sweden

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RESPONSES OF PERIPHERAL VESTIBULAR NEURONS TO ANGULAR AND LINEAR ACCELERATIONS IN THE SQUIRREL MONKEY

J M Goldberg and C Fernandez

From the Departments of Pharmacological and Physiological Sciences and of Surgery (Otolaryngology), the University of Chicago, Chicago, Ill, USA

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ct Peripheral neurons innervating semicircular can respond to constant linear accelerations Evi is presented that, in our preparation, the response actual and arises from thermal gradients introduced surgical exposure Otolith neurons do not respond n intense angular accelerations Canal plugging ies the response of the corresponding afferents to r acceleration, without obviously affecting resting / The procedure does not prevent the canals from ding to linear accelerations. The latter response, that seen in intact canals, is not due to thermal its and may be related to the mechanisms under he persistent component of barbecue nystagmus

emicircular canals are commonly thought sensors of angular accelerations, the oto-rgans sensors of linear forces This concept ot gone unchallenged Ledoux (1949) ob-d that canal neurons in the frog were sensi-o head tilt and his observations have re-/ been confirmed by Lowenstein (1972) in ly Gernandt (1950) presented evidence that esponse of canal related neurons in mam could be modified by centrifugal force on et al (1970) found that rotating linear erations could affect the discharge of canal-'d neurons in the cat vestibular nuclei and is suggested that this response provided a iological basis for the persistent component irbecue nystagmus In addition several in-gators (Benson & Barnes, 1973, Lowenstein, Young 1974) have proposed that the otolith organs might be sensitive to angular accelerations, since such accelerations could cause a torsional motion of the otolithic membrane relative to the macula

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A primary purpose of the present study was to investigate the possibilities that the mammalian semicircular canals could respond to linear accelerations, the otolith organs to angular accelerations During the course of the study, we also had occasion to investigate the response of peripheral neurons innervating canals which had been inactivated by the plugging procedure of *Money & Scott (1962)*

METHODS

Fourty four adult male squirrel monkeys (*Saimiri sciureus*), anesthetized with sodium pentobarbital (15 mg/kg), were used Body temperature was maintained at 36–38°C Surgical and recording procedures were identical to those described previously (Goldberg & Fernandez, 1971) The left vestibular nerve was exposed by removal of the posterior part of the cerebrum and a small portion of the cerebellum The nerve was covered with a 3% solution of agar–agar dissolved in physiological saline and a chamber mounted over the exposure The chamber was filled with mineral oil The microelectrode—a glass micropipette filled with 3M NaCl—was advanced by means of a screw micrometer mounted

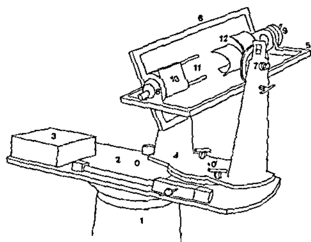


Fig 1 Diagram of stimulating device *1* rotating device *2* horizontal platform *3* ×1 000 amplifier *4* super structure mounted on guide rails As shown here, superstructure yaw axis (*0*) 1 ft from horizontal platform axis (*0*) The superstructure s outer frame (*5*) pivots around pitch axis (*7*), the inner platform (*6*) pivots around roll axis (*8*, *9*) *10* negative-capacitance preamplifier *11* rails for attaching animal head holder so that center of head coincides with intersection of superstructure's pitch, roll and yaw axes Center of head defined as intersection of interaural axis and midsagittal plane Signals pass from animal to horizontal platform via slip-ring assemblies mounted on the three superstructure axes and then pass from horizontal platform by way of a slip ring assembly in the rotating device

on the top of the chamber Temperatures within the chamber were measured with thermistors (Yellow Springs Type 511) implanted through small holes drilled in the skull and then sealed with dental wax In two experiments, thermal gradients in the chamber were controlled Two 9-ohm, 2-watt wirewound resistors served as heaters They were mounted on the inside walls of the chamber, one near the top of the mineral oil column, the other near the bottom The resistors were powered by a d c battery and the current through them was controlled by a potentiometer The superior canal was plugged in 6 of the animals A small hole was drilled in the eminentia arcuata and the exposed membranous canal compressed with a mixture of bone dust and bone wax

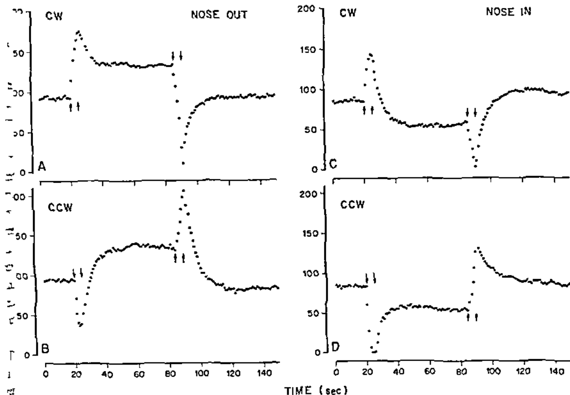
The animal was placed in a superstructure mounted on guide rails, which in turn were fixed to the horizontal platform of a rotating device (see Fig 1) The superstructure could be moved along the rails so that the animal's he[ad was] centered either over the axis of rotation or [in an] eccentric position up to 1 ft off axis The [superstructure] could also be pivoted in the superst[ructure] around pitch, roll or yaw axes By con[vention] the zero tilt position was such that the h[orizon]tal canal was in the horizontal plane [with the] animal's nose pointing towards the axis of [rota]tion The motion of the rotating device [was con]trolled by a velocity servomechanism (Model 823) Centrifugal force and sta[tic tilts] were used to study the response to linear [forces] In the centrifugal force experiments, force [trape]zoids were used with the animal 1 ft off [axis] The five periods of the trapezoid (T1 T[5) are] characterized as follows A background [velocity] $V_0 = 90°/sec$ ($\equiv 0\ 077\ g$) was maintained [during] T1 and T5 During T3, the velocity was [held at] a higher value $V_f = 360°/sec$ ($\approx 1\ 23\ g$). [In] the transition periods T2 (and T4) the [velocity] increased (decreased) in such a way th[at the] centrifugal force changed in a linear man[ner]

As a preliminary step, we examined [the re]sponse of each unit to angular acceleratio[ns and] to various head tilts Neurons innervatin[g a] particular semicircular canal were easily [identi]fied because they responded to angular ac[celera]tions when the plane of the canal was b[rought] into the plane of motion Units were con[sidered] otolith neurons if they proved unrespon[sive to] angular accelerations, but were sensitive t[o head] tilt Note that the classification scheme d[oes not] exclude the possibility that head tilts [may] also affect the discharge of canal neuro[ns In]deed, as we shall see, this was a common [occur]rence

RESULTS

Response of semicircular canal neurons to linear forces

Fig 2 illustrates the response of a posterior neuron to both angular and linear acceler[ations] Force trapezoids are used and the plane [of the] ipsilateral (left) posterior canal is in the pl[ane of] motion There are two components to th[e re]sponse The angular component is most p[romi]nent during the periods of velocity transiti[on]



²2 Response of left posterior-canal neuron to force ...zoids, posterior canal in plane of motion (90° ...alateral roll, 45° backward pitch) *A* and *B* 180° ... *C* and *D* 0° yaw In this and other figures, unless otherwise stated, T1 20 sec, T2 5 sec, T3 = 60 sec, T4 – 5 sec, T5 60 sec, arrows (or bars) mark periods of velocity (force) transition, each point, discharge rate for 1 sec period

...rses direction when the rotation is changed ... CW to CCW As expected, no reversal of ... angular component is seen when the animal ...yawed 180° with respect to the rotation ... (cf Fig 2, left and right) The second ...ponent is a response to centrifugal force ... is indicated by three facts First, the response ...ists throughout the period of constant velo... Second, there is no response reversal when rotation is changed from CW to CCW And ...d, the response is reversed from excitatory to ...ibitory when the animal is yawed 180°

...o minimize the influence of angular accelera...n, the animal was positioned so that the ...terior canal was orthogonal to the plane of ...tion (Fig 3) Again responses to centrifugal ...ce are evident in both yaw positions Em...dded in the responses are small angular ...mponents, presumably the result of a slight ...salignment of the animal The uncontaminated response to centrifugal force can be obtained by averaging the CW and CCW responses for each yaw position (Fig 3, bottom) The excitatory (bottom left) and inhibitory (bottom right) reconstructions have similar time courses and magnitudes The build-up and decline of the responses are both consistent with a first-order lag system having an approximate time constant of 5 sec These relatively slow dynamics may be taken as evidence that the response to linear accelerations involves a movement of the cupula and endolymph

Static tilts were used to characterize the directional selectivity and magnitude of the response to linear forces Studies were carried out in 47 superior-canal neurons, 48 posterior-canal neurons, and 47 horizontal-canal neurons Superior-canal neurons were activated by contralateral rolls and backward pitches, posterior-canal neurons by contralateral rolls and forward pit-

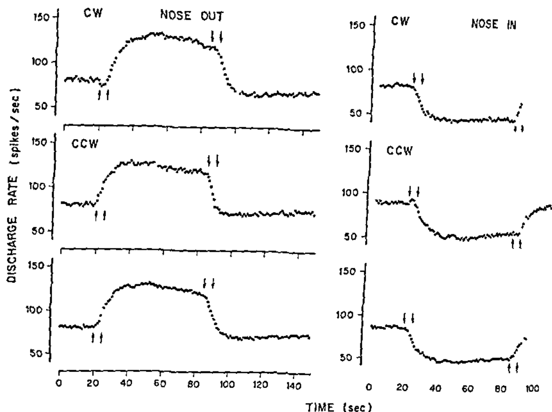


Fig 3 Response of same posterior-canal neuron as in Fig 2 to force trapezoids, animal in horizontal position *Left* 180° yaw *Right* 0° yaw

ches, and horizontal-canal neurons by ipsilateral rolls and forward pitches These rules held for over 90% of the vertical-canal neurons studied and for approximately 80% of the horizontal-canal neurons examined The response magnitudes can be expressed in terms of a sensitivity factor—the maximum response expected to a 1-*g* acceleration (Fernández et al, 1972) The mean sensitivities (±S E M) for neurons innervating the three canals were superior canal, 26 0±2 1 spikes/sec, posterior canal, 16 9±1 6 spikes/sec, and horizontal canal, 11 0±1 0 spikes/sec

The results could be explained were there a thermal gradient across the labyrinth To investigate this possibility, thermistors were implanted on the top of the superior canal and on either the vestibular or trigeminal nerves Measurements were made in 5 animals A thermal gradient of 0 5-2 0°C was observed, in all cases, the top of the canal was colder than the deeper location Undoubtedly, the gradient is due to a partial equilibration of the m in the chamber with room air The dir the gradient is consistent with the di selectivity of the responses

An attempt was made in 2 animals t the gradient by means of electric hea planted in the chamber Fig 4 present from a superior-canal unit At the sta experiment, the heaters had been adj eliminate the gradient and no respo observed (Fig 4A) The heaters were the off and a gradient of about 1 5°C develop the next several minutes The correspon sponse (Fig 4B) was typical of that more sensitive superior-canal units Heat then resumed, both the gradient and sponse reversed direction (Fig 4C) As step, the gradient was again eliminated a response considerably reduced (Fig 4D) results are consistent with Young's (1973) systematic observations and suggest th

-ured thermal gradients are adequate to in both the direction and magnitude of the -acceleration response.

nse of otolith neurons to angular rations

5 compares the response of an otolith n to angular and linear accelerations The lus was a velocity sinusoid with an accelera- magnitude of 360°/sec² With the animal ed over the axis of rotation, no response dent in either the response (Fig 5A) or histograms (Fig 5C) The lack of re- e is not attributable to an unfortunate oning of the animal relative to the plane otion Results, comparable to those ob- l in the zero-tilt position, were also seen the animal was pitched or rolled by 90° ntrast, when the animal is moved to an ric position, a clear response is observed 5B and D) The response has a frequency e that of the stimulus, as would be expected the centrifugal-force profile resulting from city sinusoid In Fig 5E, force trapezoids ed Again there is a response only in the ric position The response illustrates that neurons have relatively rapid dynamics ontrast between the response dynamics of and canal neurons can be seen by compar- g 5*E* with the reconstructed responses at ttom of Fig 3

ntially identical observations were made en otolith neurons Based on a previous ication scheme (Fernández et al, 1972), six units were assigned to the superior vesti- nerve and presumably innervated the lus The seventh was assigned to the in- nerve and most likely was a saccular nt In none of the units was a periodic re- e seen to sinusoidal angular accelerations nly hint of a response was a small increase spikes/sec) in the average discharge occur- uring the sinusoidal stimulus

nse of neurons innervating plugged canals

nimals, the superior canal was inactivated gging A total of 160 vestibular units were

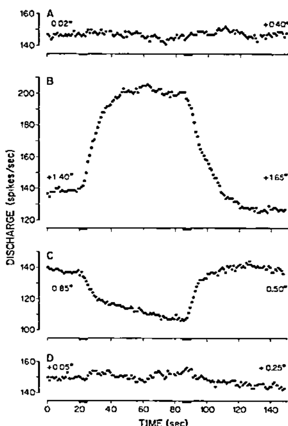


Fig 4 Response of superior-canal neuron to CW force trapezoids, animal in zero-tilt position. Temperature differences measured between trigeminal nerve and top of superior canal positive values, trigeminal nerve warmer, measurements made before and after each rotation

isolated None of the units responded to angular accelerations in the plane of the superior canal. Sixty of the units could be assigned to the horizontal or posterior canals, 53 were classified as otolith neurons, since they were not influenced by angular accelerations, but did respond in a brisk manner to rapid head tilts The remaining 47 units were considered as innervating the superior canal and, of these, 16 were studied in some detail The resting discharge of the 16 units ranged from 5 to 120 spikes/sec with a mean ($\pm$S E M) of 82 5$\pm$8 8 spikes/sec These figures are similar to those characterizing units innervating unplugged canals (Goldberg & Fernandez, 1971)

Though the units were unaffected by angular accelerations, they did respond to linear accelera-

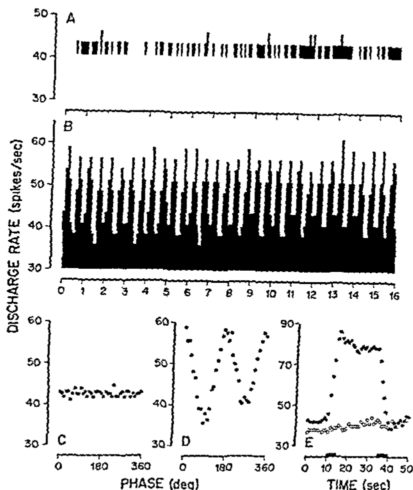
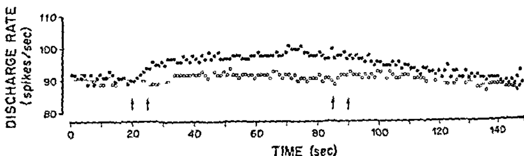


Fig 5 Response of o[…] animal in zero-tilt […] 0 257 Hz velocity […] *sponse histogram*, h[…] over rotation axis. […] histogram, head 1 h[…] scissa in units of […] (3 89 sec) size of bin, […] *C* and *D* phase his[…] sponding to *A* and *B* […] 0°, maximum CCW […] bins/cycle *E* CW […] (*T1* = *T5* = *10* sec *T2* […] T3 = 20 sec) ○ head […] rotation axis ●, head […]

tions Force trapezoids were used The animal was positioned so that the plane of the superior canal was in the plane of motion Response was studied in two positions In the first (or S1) position, the animal was rolled 90° towards the contralateral side and then pitched forward 45° The second (or S2) position was identical except that the animal was also yawed 180° In the S1 position, the animal's nose was poi[…] wards the rotation axis, in the S2 […] was pointing outwards A typical u[…] trated in Fig 6 Excitatory respons[…] tained in both S1 and S2 position[…] sponses are asymmetric, the S2 posi[…] the more effective Response dynamic[…]

In 12 of the 16 units, excitatory […]



obtained in both the S1 and S2 positions, 2 position being more effective in 13 units onse amplitudes were determined by comg the average discharge rate during the ɔth sec of T3 with the rate during T1 The S2 and S1 responses were, respectively, 1 5 and 1 9±0 6 spikes/sec

DISCUSSION

ɔnse of canal neurons to linear accelerations

semicircular canals are capable of respond- ɔ linear accelerations Our results indicate, ver, that in the monkey the response is artial, largely the result of thermal gradients duced during exposure of the vestibular and the petrous pyramid The measured al gradients are of the correct sign to in the directional selectivity of the re- se of vertical-canal neurons We need only ose, in agreement with observation, that the l aspect of each canal is colder than more al parts The situation is less clear for the ontal canal which, in our preparation, is ast exposed of the three canals Possibly for eason, the directional rules were most vari- or horizontal-canal neurons and these units in general characterized by smaller re- es Our observations do not eliminate the bility of a small respons- in the absence of nal gradients The results illustrated in Fig re based on thermal measurements made nal to the labyrinth and these, at best, can ide only an indirect indication of labyrin- temperatures Critical observations would ire the implantation of two thermistors with- single canal and this would have to be mplished without perturbing the circula of endolymph Further, the calculations of r et al (1968) would indicate that labyrin e temperatures would have to be monitored an accuracy of 0 01–0 05°C

hermal gradients would seem an unlikely e for the responses observed in cold blooded arations by Ledoux (1949) and Lowenstein '2) But here another explanation is possible It involves the supposition of a density difference between the cupula and endolymph Both Ledoux (1949) and Lowenstein (1972) used isolated preparations After death there may be an equilibration of the ionic compositions of perilymph and endolymph and this would cause the endolymph to become less dense than normal Interestingly, Lowenstein's (1972) results in the ray could be explained were the endolymph more buoyant than the cupula It must be emphasized that the required density differences are small In the squirrel monkey, for example, calculations indicate that a difference of 10^{-4} gm/cm^3 would produce in canal afferents an average response of 10 spikes/sec to a 1-*g* linear acceleration [1] Such small density differences could easily arise from experimental per turbations Indeed the differences are so small that the presence of a response in any of the preparations currently used to record vestibular-nerve activity should not, in our opinion, be taken as convincing evidence that a similar response would occur under more normal physiological conditions

Despite these uncertainties, the fact that the canals are potentially sensitive to such forces has both clinical and experimental implications First, a small and unpredictable positional nystagmus is commonly observed in seemingly normal subjects (Barber & Wright, 1973, Miyata & Igarashi, 1973) The etiology of the nystagmus is undoubtedly complex, but small density differences between the cupula and endolymph may be contributory Second, such

[1] The calculation is as follows There is a linear relation $P = K_D\alpha$, between a constant angular acceleration α and the resulting pressure differential across the cupula P K_D can be deduced from canal dimensions (Oman & Young, 1972) Suppose the canal were sensitive to linear accelerations because of a density difference $\Delta\rho$ between the cupula and endolymph Equating the pressure differentials arising from an angular and a 1 *g* linear acceleration gives $\Delta\rho = K_D\alpha/gh$ h being the width of the cupula The response s of a representative canal afferent to the linear acceleration is $s = K_s\alpha$ K_s is the afferent s sensitivity to angular accelerations Hence $\Delta\rho = K_Ds/K_sgh$ Typical values are $K_D = 5 \times 10^{-4}$ dyne cm^2 per deg sec^2 and $h = 2\ 5 \times 10^{-2}$ cm (Igarashi, 1966) also $K_s = 2$ spikes/sec per degree/sec^2 (Goldberg & Fernandez, 1971) The proportionality constant between $\Delta\rho$ and s is found to be near 10^{-5} g cm^3 per spikes/sec

density differences are implicated in the larger *positional nystagmus that occurs following drug* intoxication (Money & Myles, 1974) And third, in studies of central vestibular neurons, it is usually assumed that units responding to static tilts receive an otolith input The most common pattern of convergence described in the vestibular nuclei involves individual neurons receiving a canal and a presumed otolith input (Duensing & Schaefer, 1959, Markham, 1972) The conclusion is based on the observation that the same neuron responds to angular accelerations and slow head tilts Both the conclusion, and the assumption upon which it is based, may be unwarranted

Response of otolith neurons to angular accelerations

Our methods for categorizing peripheral vestibular neurons depend on the assumption that otolith neurons do not respond to angular accelerations That this assumption is correct, and is not simply based on an arbitrary classification scheme, is indicated by several observations First, virtually all units responsive to natural vestibular stimulation can easily be classified Second, units responding to angular accelera tions only do so when the appropriate canal plane has a component in the plane of motion It is unlikely that otolith neurons, assuming that they were responsive to angular accelerations, would exhibit directional selectivities precisely correlated with individual canal planes Third, otolith units are most commonly encountered when the recording electrode is aimed for the posterior part of the superior vestibular nerve or the ventral part of the inferior vestibular nerve Anatomical studies (Gacek, 1969, Sando et al, 1972) demonstrate that utricular afferents are concentrated in the former region, saccular afferents in the latter region Fourth, canal and otolith neurons can be independently disting uished on the basis of their response dynamics *Canal neurons have slow dynamics* (Fig 3), characterized by a first order time constant of about 5 sec (Fernandez & Goldberg, 1971) Otolith neurons have rapid dynamics (Fig 5*E*) and have a corresponding time constant esti mated to be on the order of 001-[illegible] (Goldberg & Fernandez, 1974)

It is not entirely clear why otoli[illegible] not respond to even intense angular [illegible] tions *A priori* it would seem reasonable [illegible] pose that such accelerations would [illegible] torsional movement of the otolithic me[illegible] Conceivably the torsional rigidity of [illegible] brane might be high Alternately the lack [illegible] sponse may reflect the geometric [illegible] the hair cells Given the morphological p[illegible] tion of the maculae (Lindeman, 1969) [illegible] movements would deflect most sensor[illegible] bundles in a direction perpendicular to [illegible] polarization axes Presumably such [illegible] deflections are relatively ineffective

Response of neurons innervating p[illegible]

Canal plugging, consistent with behav[illegible] servations (Money & Scott, 1962), [illegible] the response of canal neurons to ang[illegible] tions without having an obvious ef[illegible] activity The procedure does not, howe[illegible] vent the canals from responding to linea[illegible] The responses cannot be explained by [illegible] ence of thermal gradients A caloric [illegible] the response to angular accelerations, [illegible] the circulation of endolymph and this s[illegible] prevented by the plugging procedure [illegible] another way, an occlusion of the mem[illegible] duct which abolishes responses to [illegible] accelerations should also eliminate ca[illegible] sponses Further, caloric responses sh[illegible] bidirectional That is, if linear accelera[illegible] one direction are excitatory, those in th[illegible] site direction should be inhibitory In [illegible] our responses, though asymmetric, we[illegible] directional The unidirectionality wou[illegible] seem to rule out any simple mechanism ba[illegible] a difference in density between the cup[illegible] endolymph

These observations are relevant to the e[illegible] of the persistent component of barbecue [illegible] mus Correia & Money (1970) plugged [illegible] canals in cats and observed only a slight r[illegible] tion in the nystagmus It was concluded th[illegible] persistent component could be maintained [illegible]

otolith activation The conclusion was based the assumption, at variance with present ts, that plugging totally inactivates canals nson & Bodin (1966) and Steer (1968) attempted to explain the persistent component by ning that linear forces deform the membranous canal and thereby produce a pressure ential across the cupula In an intact canal, nstant force will be ineffective since the ure on the two sides of the cupula will ize within a few msec A rotating force , however, lead by a roller pump action to tained deflection Also, a constant force on a plugged canal might be effective, occlusion of the membranous duct would nt pressure equalization Whether this or a d mechanism underlies the unidirectional symmetric responses obtained from plugged s cannot be decided Of interest, though, is act that Benson et al (1970), in their study e influence of rotating linear accelerations ntral neurons, found responses which were unidirectional and asymmetric

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ZUSAMMENFASSUNG

iere Neuronen die den Bogengang innervieren, n auf gleichbleibende lineare Beschleunigung rea Es wird gezeigt dass diese Reizbeantwortung in m Praparat kunstlich ist, und dass sie aus den aschen Gradienten entsteht, die auf die chirurgische gung zuruckzufuhren sind Otolithneuronen sind durch starke Winkelbeschleunigung nicht erregbar Tamponieren des Bogengangs loscht die Reiz wortung der entsprechenden Afferenten auf die lbeschleunigung ohne dass es eine deutliche Wirauf die Ruhetatigkeit hat Das Verfahren loscht da nicht die Reizbeantwortung des Bogengangs auf e Beschleunigung Anders als bei unoperierten ngängen wird diese Beantwortung nicht von den ischen Gradienten veranlasst und konnte mit dem anismus der beharrlichen Komponente des ' bar Nystagmus zusammenhangen

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J M Goldberg Ph D
Dept of Pharmacological and Physiological Sci[illegible]
University of Chicago
951 E 58th Street
Chicago, Ill 60637, USA

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ANALYSIS OF CURVES Na-24 EFFLUX FROM MEMBRANE LABYRINTH CONTAINING STRIA VASCULARIS

Z Mnich

From the Department of Biophysics, and the Institute of Physiology and Biochemistry, Bialystok Medical School, Bialystok, Poland

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The efflux of Na 24 was investigated in isolated hs from a membrane section containing stria ris and spiral ligament in artificial endolymph ı The efflux curves were calculated and compart analyses were made The theoretical possibility cchange of sodium between compartments con n different ways was considered The calculated of rate constant of transmembranal sodium fluxes 282 ± 0 0052 min ¹ and the half time of the ex- of sodium between intracellular compartments is 4 53 min The distribution of sodium in tissue commonly applied simplification gives 90 % Na+ extracellular compartment and 3 % Na+ in the lular compartment of the total tissue sodium The possibility of measuring the rate constant membranal electrolyte fluxes in this tissue seems iging for future investigation of the mechanism h they are transported

ariability of the electrolyte composition of ar endolymph as an extracellular fluid, s potential significance (Békesy, 1952) are ause of much speculation Investigators obtained a great deal of experimental data rning these phenomena in various experi- il conditions (Konishi et al , 1966, Kuijpers nting, 1970, Prazma, 1969, Sellick & John 1972 Silverstein, 1970, Tanaka & Brown,

s well known that the distribution of elec- es between adjoining spaces, and the kinet their exchange, are the basis on which the ical phenomena are explained (Hodgkin, › So far, however, we have had no kinetics about the exchange of electrolytes between tria vascularis cells and endolymph

The present work attempts to estimate some kinetic parameters of sodium transport, on the basis of efflux curves

METHOD

The experiments were carried out on an isolated section of a guinea pig membrane labyrinth which contained stria vascularis and spiral liga ment The animals were decapitated, the skulls quickly opened and the cochlea taken out In artif- icial endolymph (4°C) a part of the membrane la- byrinth which contained stria vascularis and spiral ligament was then isolated The whole procedure of preparation was done using a stereoscopic microscope (12×) and lasted about 20 min Subsequently the tissue was incubated in a medium similar to endolymph (artificial endo- lymph) which contained 144 mM K^+, 5 mM Na^+, 3 mM Ca^{++}, 2 mM Mg^{++}, 135 mM Cl 15 mM HCO_3^-, 1 2 mM $H_2PO_4^-$, and 6 mM glucose, at a temperature of 37,5°C and pH 7 35 The total volume of incubation medium was 3 0 ml and it was circulated by a constant stream of mixture (97 % O_2, 3 % CO_2) The incubation medium contained Na 24, specific activity was mCi/ml After the incubation, which lasted about 40 min, the preparation was rinsed Mullins & Frumento, 1963) in a wash-out solution, also artificial endolymph of exactly the same com position, but without isotopes In this way, samples were obtained with Na-24 which ha

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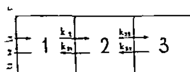
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J M Goldberg Ph D
Dept of Pharmacological and Physiological S...
University of Chicago
951 E 58th Street
Chicago Ill 60637, USA

antitative expression of kinetics of sodium
nge at the cell membrane level (Zierler,
Van Liew, 1967) Furthermore, if we are
empt to combine the kinetics of sodium
nge between stria vascularis cells and endo-
with resting endolymphatic potential, we
bear in mind several possible means of
g separate compartments obtained from
mental three-component curves
nsider for example the following cases
the work of Levin & Patlak (1972) The
three-compartment model series in which
ree compartments are connected together



values of the particular rate constants ex-
e between compartments are given by
tion

$$-\sum_{1}^{3} B_i\lambda_i$$

$$\frac{\sum_{1}^{3} B_i\lambda_i^2}{\sum_{1}^{3} B_i\lambda_i}$$

$$\frac{\sum_{1}^{3} B_i\lambda_i^3}{\sum_{1}^{3} B_i\lambda_i^2}$$

$$k_{11}-k_{10}$$

$$\frac{1}{k_{12}}\left[\frac{\sum_{1}^{3} B_i\lambda_i^4}{\sum_{1}^{3} B_i\lambda_i^2}-k_{11}^2\right]$$

$$\frac{1}{k_{12}k_{21}}\left[\frac{\sum_{1}^{3} B_i\lambda_i^5}{\sum_{1}^{3} B_i\lambda_i^2}-k_{11}^3-2k_{11}k_{12}k_{21}\right]$$

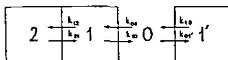
$$k_{22}-k_{21}$$

$$k_{32}=k_{23}k_{12}k_{21}\left[\frac{\sum_{1}^{3} B_i\lambda_i^6}{\sum_{1}^{3} B_i\lambda_i^2}-k_{11}^4-k_{12}k_{21}\right.$$

$$\left.\times(3k_{11}^2+2k_{11}k_{22}+k_{12}k_{22}+k_{22}^2)\right]$$

As described by Levin & Patlak (1972), in the flow from medium to tissue, coefficient B_i is negative The efflux from tissue to medium is described by the same values (B_i), except that they are positive Thus in the equation for k_{01}, the sign is plus

One should note that the calculated values for k_{ij} here describe the kinetics of exchange but we cannot consider their values as a ratio constant of the efflux between cellular space and medium The reason for this divergence is assumed by considering the model equality of compartments It is possible to obtain more adequate values for k_{ij} by taking into consideration the siz of the compartments A second possibility is the coexistence of two compartments in series and one in parallel



The particular values of rate constant exchange between the compartments are given

$$k_{01}-(B_1\lambda_1+B_2\lambda_2)$$

$$k_{10}=\frac{B_1\lambda_1^2+B_2\lambda_2^2}{B_1\lambda_1+B_2\lambda_2}$$

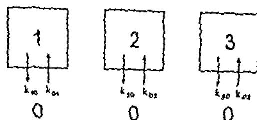
$$k_{11}=\frac{B_1\lambda_1^3+B_2\lambda_2^3}{B_1\lambda_1^2+B_2\lambda_2^2}$$

$$k_{11}=\frac{B_1B_2\lambda_1\lambda_2(\lambda_1-\lambda_2)^2}{B(_1\lambda_1+B_2\lambda_2)(B_1\lambda_1^2+B_2\lambda_2^2)}$$

$$k_{21}=\frac{(B_1\lambda_1+B_2\lambda_2)\lambda_1\lambda_2}{B_1\lambda_1^2+B_2\lambda_2^2}$$

$$k_{1'0}=\lambda_3 \quad k_{01'}=B_3\lambda_3$$

A third possibility is a three-compartment model directly connected with medium



The values of k_{10}, k_{20}, k_{30}, are given immediately by λ_1, λ_2 and λ_3, though k_{01}, k_{02}, k_{03} are given by $B_1\lambda_1$, $B_2\lambda_2$, $B_3\lambda_3$. In this case the rate constant for each particular compartment is equal to the exchange constants k_{ij} of the cellular membrane. The above model may also be an approximation in the case of three kinds of cells which have differing kinetics of exchange, assuming that the diffusion process in extracellular space is very quick. Another possible interpretation of this model is that one compartment is extracellular space, when geometry and other conditions are the cause of small exchange between cells and extracellular space.

The fourth possibility is thus compartment 1 is the extracellular space and compartments 2 and 3 represent two different kinds of cells (Fig 5). One can see that the exchange between these cells and the medium is possible only via the extracellular space. Mathematically this model may be described by equations

$$\frac{dC_1}{dt} = k_{01}C_0 + k_{21}C_2 + k_{31}C_3 - (k_{10} + k_{12} + k_{13})C_1$$

$$\frac{dC_2}{dt} = k_{12}C_1 - k_{21}C_2$$

$$\frac{dC_3}{dt} = k_{13}C_1 - k_{31}C_3$$

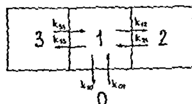


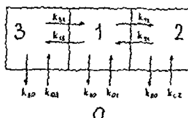
Fig 5

The general solution of the above system ... tions has the form

$$\frac{C_1 + C_2 + C_3}{C_0} = B_0 + B_1\exp(-\lambda_1 t) + B_2\exp(-\lambda_2 t) + B_3 \exp ...$$

where C_1, C_2, C_3 represent the sodium ... tration in the compartments and C_0 ... medium. Following Levin & Patlak (... same expression was obtained for $k_{...}$ ... though for the next coefficient other ... sions were obtained which do not give ... solution for k_{ij}

A more complicated but more probable ... is given below



Mathematically this model descri... system for the differential equations

$$\frac{dC_1}{dt} = k_{01}C_0 + k_{21}C_2 + k_{31}C_3 - (k_{10} + k ...$$

$$\frac{dC_2}{dt} = k_{02}C_0 + k_{12}C_1 - (k_{20} + k_{21})C_2$$

$$\frac{dC_3}{dt} = k_{03}C_0 + k_{13}C_1 - (k_{30} + k_{31})C_3$$

The solution of these equations is ... plicated than the one before last

Irrespective of the theoretical possi... difficulties presented above in exact c... of the particular rate constant of ex... tween particular compartments, one ... first approximation assume that the ... rate constants λ_1, λ_2 concern the efflux ... cellular sodium and the slow rate co... represents the transmembrane fluxes o... (Brading & Jones, 1969, Brading, 197... strom, 1973). In such an interpretatio... vestigated tissue about 90% of Na-24 ... buted in extracellular space and 7% ...

:llular Taking into account Huxley's cor- factor (Huxley, 1960)
lified by Brading (1971) and Wahlström the intracellular compartment contains 3 % Na of the total tissue content, the ing 7 % is probably very rapidly exchange- dium located on the surface of the tissue tained value of the rate constant of trans- anal effluxes is equal 0 0282 min^{-1} in endo- and is similar to that obtained by Wahl- (1973) from rat portal vein—0 0280 min^{-1} lution like Krebs, and by Brading (1971) *aenia coli* in Krebs solution This agree- puzzling because it exists in very different of medium in this peculiar tissue
ould appear that some explanation con- the mechanism of electrolyte transport n stria vascularis cells and endolymph give more precise measurements trans- anal effluxes in various experimental con- , even if at the outset we were to assume dure similar to that used in morphologi- omogeneous tissue

ZUSAMMENFASSUNG

ı kunstlichen Endolymphe-Milieu wurde die Aus ng des Na 24 aus dem die Stria vascularis und mentum spirale enthaltenden Teil des Membran ıes untersucht Es wurden gewisse theoretische keiten des Natriumaustausches zwischen den er Teilen diskutiert. Der berechnete Wert der ındigkeitskonstante der Natriumauswanderung Zellen des untersuchten Gewebes betragt 0 0282 ± nın $^{-1}$ Die Halbwertzeit des Natriumaustausches n intrazellularen Teil betragt 24 min. Der ge Natriumabbau im Gewebe gibt bei den fur ge h angewandten Vereinfachungen 90 % im extra n Teil lokalisiertes Natrium und ca 3 % Gesamt natrium im intrazellularen Teil Die Moglichkeit essung der Geschwindigkeitskonstante des trans ınen Elektrolytenstromes in diesem Gewebe neue Forschungsmoglichkeiten fur den Austausch ıismus der Elektrolyten zwischen den Zellen dieses les Membranlabyrinthes und der Endolymphe

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Z Mnich, M D
Dept of Biophysics
Institute of Physiology and Biochemistry
Medical School of Bialystok
15 222 Bialystok
Poland

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SURGICAL ANATOMY OF THE GENICULATE GANGLION

M Dobozi

From the Department of Otolaryngology, University of Zurich Zurich Switzerland

(Received May 28 1974)

Abstract The position and the average dimensions of the geniculate ganglion have been measured with the Zeiss screw micrometer in 24 temporal bones obtained from the histological collection of the ENT department of the University of Zurich The geniculate ganglion has been found to lie like a cup over the anterior portion of the genu of the VII nerve Its dimensions have shown only minimal variations (standard deviation of average length, height and width less than 10%) Surgical resection of the anterior third of the genu of VII nerve has been shown to include 90% of the ganglion cells but not to endanger the motor fibres These surgical measurements are the basis for performing a resection of the geniculate ganglion in cases of geniculate neuralgia

The geniculate ganglion is a complex system containing ganglion cells of the sensory (taste) fibres of the greater superficial petrosal nerve and corda tympani as well as the preganglionic secretory fibres for the sphenopalatine ganglion According to Kure & Sano (1936) the geniculate ganglion also contains other sensory ganglion cells which are not related to the function of taste but are believed to be responsible for the pain occurring in petrosal neuralgia Clinical experience (Fisch, 1973) has shown that excision of the geniculate ganglion gives better long term results in the relief of geniculate neuralgia than does dissection or excision of the greater superficial petrosal nerve The present study was undertaken with the aim of defining the exact topographical position of the geniculate ganglion in order to permit the surgical excision of this structure without damaging the neighbouring motor fibres of the facial nerve

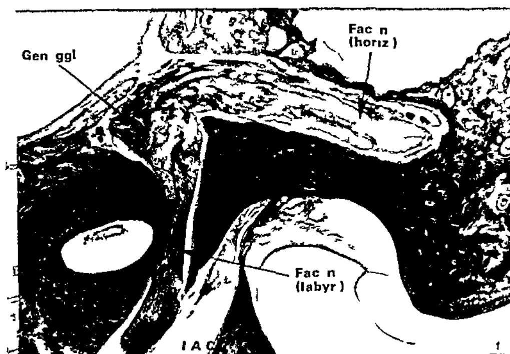
MATERIAL AND METHOD

The topography of the geniculate ga established by measurements obt histological sections of the tem collection of the ENT-department University of Zurich Twenty four bones belonging to patients ranging from 11 to 82 years were examined temporal bones were cut in a horiz 13 in a vertical plane The average thi the celloidin-embedded section was The measurements were performed wi screw micrometer ocular

RESULTS

In all examined specimens the ganglion cells were found lying like a the anterior portion of the external gen facial nerve (Figs 1 and 2) Only were a few scattered ganglion cells se mal to the genu along the labyrin meatal portion of VII nerve In fiv 24 specimens (i e 20% of the cases) the part of the ganglion was lying free in dehiscence, directly under the dura of the cranial fossa

The average dimensions of the g ganglion are shown in Fig 3 In the h plane the ganglion is triangular in shape an average length of 1 09 mm (Fig 3a) section through the ganglion shows t





Labyrinth ne and horizontal port on of the facial w h gen culate gangl on (H E. 10)
Genu of the fac al nerve Gen ggl geniculate on Fac n facial nerve (labyr) labyrinthine part of the fac al nerve (horiz.) horizontal part of the facial nerve *IAC* nternal aud tory canal *GSPN* greater superficial petrosal nerve (H E 60)

average width of this structure is 0 76 mm with an average height of 0 6 to 0 8 mm The posterior and most proximal part of the ganglion overlaps for a short extent the motor fibres (Fig 3*b*) This overlapping portion of the ganglion is, however, so thin that it can be neglected for surgical purposes This is particularly true in view of the fact that according to the investigations of Kure & Sano (1936) the most important sensible ganglion cells are concentrated in the anterior portion of the ganglion just before the origin of the greater superficial petrosal nerve

According to the measurements presented (Fig 4) surgical resection of the frontal third of the facialis genu permits excision of the majority of the ganglion cells without any danger to the motor fibres In many instances the line of division between geniculate ganglion cells and motor fibres can be identified using the operating microscope In those instances where this division is not visible, resection of the anterior third of the genu of the VII nerve permits excision of more than 90% of the ganglion cells including all those which are probably responsible for the pain related to geniculate neuralgia

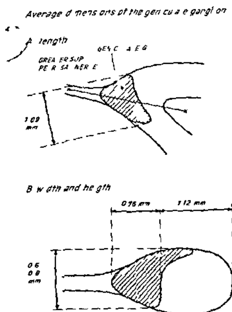


Fig 3

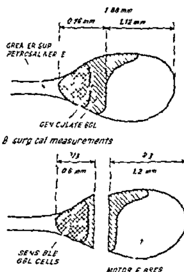


Fig 4

CONCLUSIONS

Histological measurements of the ge ganglion have shown that this structur the frontal third of the genu of the nerve The dimensions of the gangl only slightly since the standard de found for the average length, width an of the structures was in all instances l 10% For surgical purposes, 90% ganglion cells of the geniculate ganglior excised by removing the anterior third genu

RÉSUMÉ

La position et les dimensions moyennes du g geniculé ont été mesurées sur 24 rochers Le g geniculé se presente en forme de coupe au-des partie anterieure du genou du nerf facial Ses di n'ont montré que des variations minimes (l standard de la longueur, de la largeur et de la moyenne inférieure à 10%) Une section chirur tiers antérieur du genou du nerf facial compr des cellules ganglionnaires et évite tout dom fibres motrices Ces mesures constituent la ba résection du ganglion génicule dans les n névralgies géniculées

ZUSAMMENFASSUNG

Anhand von 24 histologisch verarbeiteten Fel aus der Sammlung der Hals Nasen-Ohren-Kl

sitat Zurich wurde die Topographie des on geniculi untersucht Ziel der Studie war, Lage ausdehnung der Ganglienzellen im Bereich des sknies zu lokalisieren Das Ganglion geniculi sitzt l auf dem Facialisknie Dabei zeigen Form und hnung des Ganglions im Vergleich zum Gesamthnitt des Nerven von Fall zu Fall nur geringe chungen (unter 10 % in allen Richtungen) Eine gische Resektion des ventralen Drittels des sknies gewahrleistet aufgrund der vorliegenden uchung die Entfernung von ca 90 % der Ganglienohne Gefahrdung der motorischen Facialisfasern enntnis dieser Verhaltnisse bildet die Grundlage zur gischen Behandlung von Neuralgien des Ganglion —li

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M Dobo-i, M D
Dept of Otolaryngology
Kantonsspital
8005 Zurich
Swit erland

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AN INTERACTIVE PROGRAM FOR THE ANALYSIS OF ENG TRACINGS

E Anzaldi and E Mira

From Centro di Studio per l'Interazione Operatore Calcolatore of the C N R and from the Otorhinolaryngological Clinic, University of Pavia, Pavia Italy

(Received September 23, 1974)

Abstract An interactive program for clinical and research evaluation of vestibular nystagmus has been developed Because of the interactive structure of the program, the clinician can make immediate decisions on the interpretation of controversial aspects of the nystagmogram, therefore even the more irregular and noisy ENG tracings, frequently obtained from pathological subjects, can be easily processed In this way, speed and accuracy of computer processing are coupled with intelligent activity and critical judgement of the clinical evaluation The program can be implemented on a general purpose minicomputer and no specific technical training is required for its use The structure of the program and the operations performed by main subsystems are discussed in detail Examples of ENG tracings processed with the program are reported

The analysis of vestibular nystagmus recorded by electronystagmography (ENG) is becoming the preferred procedure for the valuation of vestibular function in many clinical and research investigations

However, direct manual measurements of ...ious elements of the nystagmic response, ... as number of frequency of nystagmus ...ats, total angular displacement of eyes (Gesamt-amplitude), slow phase angular velocity, are very cumbersome and time-consuming

For this reason several analog and computer methods have been proposed for automatic or semi automatic analysis of ENG records both methods have advantages and disadvantages

Analog devices (Henriksson, 1955, Benson & Stuart, 1966, Guedry & Turnipseed, 1968, Voots, 1969, Blauert, 1972, Max et al, 1973, McClure et al, 1973) are relatively unexpensive and easy to use, but they are scarcely ... their analysis is limited to single elemen... nystagmic response and may give ri... errors due to integration of noise from... sources or to lack in distinguishing n... from random eye movements or arti...

Digitized data processing systems ... complex and expensive and require ... computer facilities and well trained ... on the other hand they give a detailed ... of the various elements of the nyst... sponse, can automatically recognize ... ticular ENG characteristics and elimina... biological or electronic artifacts, fin... can be more easily modified in relati... specific requests of the clinician

In recent years many authors (Herb... 1968 Matz et al, 1970, Loth et al, 1... & Young, 1971, Anzaldi et al, 1972, 1973, Nagle & Anderson, 1972, Om... 1973, Schulder et al, 1973, Ward, 19... developed several programs for the an... nystagmus which perform this operat... fully automatic way This kind of p... gives excellent results if applied to select... records, presenting an even and r... sequence of beats with well defined s... fast phases and rarely interrupted by ... This is the case of optokinetic nystagm... vestibular nystagmus from normal subje... mitted to particular stimulations (e g pr... constant accelerations, sinusoidal tests) for research purposes On the contrary tracings obtained from caloric or rot... tests performed in clinical setting, esp...

hological subjects, are often very irregular
eir analysis is frequently difficult even
well trained clinician, care must be taken
ognizing uncontrolled artifacts or noise
arious sources, in detecting the beginning
e end of the nystagmic response or the
d in nystagmus direction, in identifying,
ing and classifying peculiar aspects of
tracing which may have some diagnostic
ance (pauses, dysrhythmias, square waves,
ar movements, etc.) (Jongkees, 1973).

e of the programs so far developed can
automatically these interpretation prob-
nd this is the primary reason for limiting
mputer analysis of nystagmus in clinical
e. In fact, automatic processing of ENG
s can be performed only by using very
x programs, based on the more sophisti-
methods of pattern recognition.

alternative solution is offered by an inter-
approach (Kamal, 1973): these systems
omputational algorithms for detecting
ng and classifying nystagmus beats but,
ence of anomalies or irregularities of the
tracing, they leave to the operator the
f their interpretation. In this way we
a very useful instrument, in which the
and the accuracy of computer analysis
upled with intelligent activity and critical
ent of the clinician (Anzaldi et al., 1973).

paper describes an interactive program
alysis of nystagmus (TAIS) recently devel-
t the Centro per l'Interazione Operatore
atore and now currently in use at the
nolaryngological Clinic of the University
a.

interactive pattern analysis system can
operate in line with data acquisition,
se of the time required by the operator
aluating and correlating the clinical data,
mparing different samples of the ENG
g, for making decisions in order to process
ing tracts of the nystagmograms on the
of informations previously acquired. We
therefore developed an off-line system
rising different phases: vestibular examina-
and recording of vestibular nystagmus,
both on paper and on magnetic tape, analog-to-digital conversion and storing of the data in the mass-memory of the computer, visualization of the nystagmogram on the computer display and its analysis, evaluation and printout of the results.

Both for clinical and research purposes we perform different vestibular test, caloric (Fitzgerald-Hallpike, Veits) or rotational (post-rotational or per-rotational tests with different velocity profiles).

Ocular movements in the horizontal plane are recorded by means of two bitemporal silver-silver chloride skin electrodes (Beckman type 3503), with a ground electrode placed on the forehead. The electrodes are connected with a d.c. amplifier (Beckman Dynograph Type R) and the stimulation and nystagmus signals are displayed simultaneously on a polygraph; the output of the amplifier is also coupled with a four-channel FM tape recorder (Ampex PR 500). One of these channels is used for recording a control signal which marks the beginning and the end of nystagmus registration and can be utilized in the subsequent A/D conversion for synchronizing purposes, another channel is used for recording the duration of the stimulus in caloric tests or the chair velocity in rotational tests.

In electro-oculography the relationship between ocular displacement (θ) and the variation of the corneoretinal potential detected by the electrodes (V) is linear up to 30° to each side of midposition; therefore, for correctly recording larger eye movements, which are often present at the beginning of post-rotational nystagmus, we must construct the function $V = V(\theta)$. For this reason, each vestibular stimulation is preceded by a complex calibration procedure, requiring a series of ocular displacements of different amplitude ($\pm 10°$, $\pm 20°$, $\pm 30°$, $\pm 40°$). Processing of nystagmographic data is performed by a general purpose minicomputer (Laben 70, with 16K, 16-bit words and 1.5 μsec cycle time), equipped for the analog-to-digital conversion and for the graphic presentation of the output (display-terminal Tektronix 4010).

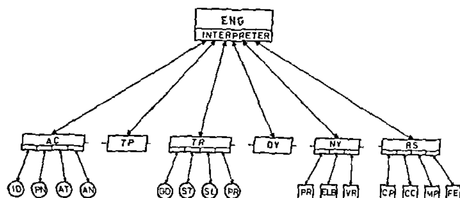


Fig 1 General s
the program TAIS

The program for the analysis of nystagmus is written in FORTRAN IV and, for input-output operations in Assembler As shown in the block diagram of Fig 1, the program (TAIS) has a tree structure, composed of a main program and several subsystems, in which every branch can be activated at operator request

AC data acquisition and retrieval,
TR analysis of calibration tracing
NY analysis of nystagmus tracing,
TP magnetic tape drivers
DY graphic input-output drivers,
RS results presentation

On the basis of the operator's requests, the main program activates a selected subsystem which, in turn, activates a series of routines, each performing a specific task

In the following pages we will give a
tion of the main subsystems, omitting
DY, which are only service subsystems

AC analog nystagmographic data,
on magnetic tape, are digitized and sto
mass memory (9 Track magnetic tape
computer The highest frequency in the
spectrum of vestibular nystagmus rarely
20 Hz and therefore the signal is an
filtered at 25 Hz The sampling rate
In order to filing the digitized data, each
mic tracing is coupled with various infor
accepted as additional inputs by the
(e g patient data, data and number of
examination type of stimulation, oth
references needed for the otoneurolog
nosis)

TR this program performs the a
the calibration tracing and calculates

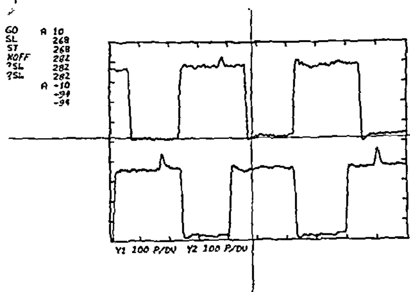


Fig 2 Measurement of
levels corresponding to
ment of ± 10 degrees

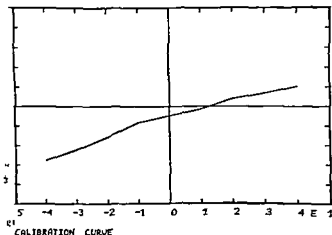


Fig 3 Display presentation of the calibration curve

...V=V (ϑ) Calibration eye movements, ...ially in pathological subjects, are often ...ilar and their anomalies may have im...nt diagnostic significance (Haring & ...ons, 1973) for these reasons, in our inter... program the analysis of these move... and the measure of potential differences ...ponding to eye displacements are tasks ...ed to the human operator Calibration ...1 is presented step-by step on the computer ...y and the clinician marks the different ...tial levels using a graphic cursor (Fig 2) ... values so measured on the vertical axis ...emorized and, at the end of this phase, ...libration curve is presented on the display (Fig 3) Moreover, if non linearities are present in the calibration, they are automatically corrected during the subsequent analysis of the nystagmus

NY This is the specific program for analysis of ENG tracing The tracing is presented step-by step on the display, on the left side of the display is also presented a menu of the routines which can be activated by the operator This operation is performed by typing the code of the selected routine in response to the question mark presented by the computer So, the operator can detect and mark, using the graphic cursor, the beginning (BG) and the end (ST) of the response, the transition from the first to the

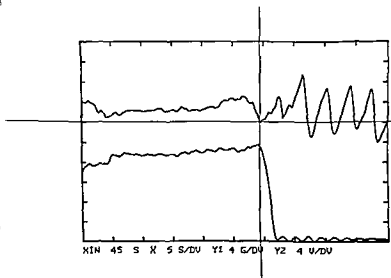


Fig 4 Determination of the beginning of a post rotational nystagmic response The lower trace shows the chair velocity

test (Fig 4) The lower trace of the presents the stimulation signal (VD) (Fig at operator request (VH), the follow of the nystagmus record (Fig 6)

During this phase of processing the can detect the artifacts present in the tracing these artifacts can be manu nated (IN) or, if the clinician attrib some diagnostic significance, can be (CM) and retrieved

In the next phase of elaboration, the activates the routine for automatically ing nystagmus beats (GO) The composing this routine (NIST, Fig 5) described in detail elsewhere (Anzaldi & 1972) on the basis of the nystagmus previously defined by the operator, the recognizes the first slow phase eye every point of the input signal is compa the preceding and the difference is and stored Whenever a reversal in the of eye movement occurs, the progra distinguish between a fast phase or a eye movement if this reversal is obser or more consecutive samples and if its exceed a threshold value, the program that a saccade has occurred The thresh (usually of 1 or 2 deg) is prefixed by the activating the routine SG and may ch the basis of the characteristics of th tracing under examination At the end analysis, nystagmus beats so detected a

Fig 5 Flow chart of the algorithm NIST

second phase (F1–I2) of nystagmus in a post-rotational test or the reversal of nystagmus direction in the different periods of a sinusoidal

Fig 6 Display presentation of a post rotational nystagmus resp cessed by subsystem NY Nystag automatically recognized have be by vertical bars In the upper rig an artifact (square wave) has be ally eliminated

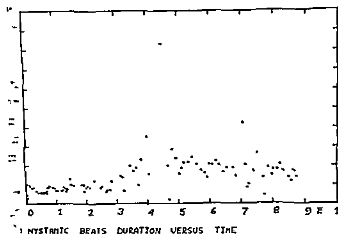


Fig 7. Display presentation of the time course of the duration of nystagmus beats in a post rotational test

ally marked on the display by a series -tical bars (Fig 6) In this way the operator recheck the validity of the recognizing ition performed by the computer and make er corrections, e g including (AD) or ving (DL) single beats or re-examining a le of the ENG record using different thresh- alues (RP)

e output of the subsystem NY is a synthetic iption of the nystagmic response, each gmus beat being defined only by its end , the response coded in this way is punched paper tape together with the data for the fication of ENG tracing, clinical informa- and operator comments The punched s retrieved and used for further elaborations nystagmus (statistical analysis, correlation coefficients, frequency histograms, etc), all performed automatically by the computer

RS this subsystem, operating on the output of the subsystem NY, gives automatically the values of the conventional elements of the nystagmic response, i e duration, number of beats, total eye displacement (Gesamt amplitude), and a running time display of the duration (Fig 7), amplitude and frequency of nystagmus beats RS can also perform a more complex elaboration of nystagmus calculating cumulative slow phase eye position (Fig 8) and slow phase velocity (Fig 9) (Anzaldi & Boselli, 1973)

In presence of spontaneous nystagmus, the operator can manually correct the automatic processing of the response, by typing the required instructions

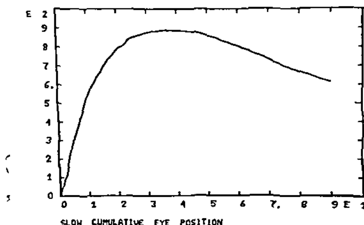


Fig 8 Display presentation of the slow cumulative eye position in a post rotational test.

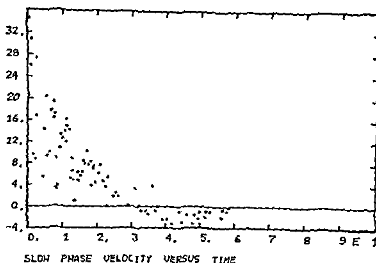


Fig 9 Display presentation of t course of slow phase velocity r rotational test

The program system comprises other additional subsystems (PL and PS) for automatically calculating the parameters of the vestibulo-ocular reflex of the subjects under examination, on the basis of a mathematical model proposed by Schmid (1970, 1974) these subsystems are not described in detail in this paper, being used at present only for research purposes

TAIS interactive program has been used over two years for currently processing ENG tracings recorded at the Otorhinolaryngological Clinic of the University of Pavia systematic comparisons show a good correspondance between the results obtained from hand-scored and automatically-scored records In our opinion, the eliability of the program is mainly due to its nteractive form, which consents a strict and continuous partnership between the clinician and the computer and therefore markedly reduces the occurrence of errors For research purposes, our digital computing technique provides a rapid and precise method for evaluating many aspects of the nystagmic response and give much quantitative information about correlations existing between the various elements of the nystagmus this information can be important for the understanding of the intrinsic mechanisms that produce nystagmus

In clinical setting, TAIS interactive system is effective and reliable in processing ENG records, even in the case of irregular and noisy tracings obtained from pathological subjects, the complete analysis of caloric or rotational te be performed in about 10 minutes.

No specific technical background in t instruments or in computer usage is re to the operator, moreover the tree-struc the program, comprising a series of sub with limited memory occupation, allo use of a general purpose minicomputer f reasons the TAIS interactive system especially suitable for clinics and labo which do not have complex and expensi puter installations

RÉSUMÉ

Un programme interactive pour l analyse du ny vestibulaire a été developpé En raison de cette s interactive, le programme offre au clinicien la p d'intervenir directement pour l interpretation de moins clairs du nystagmogramme (détermin début et de la fin de la résponse nystagmique instants d inversion du nystagmus éliminat artefacts) de cette façon même les tracés les pl guliers et compliqués, tels qu'on les obtient fréqu dans la routine clinique à partir de sujets patholo peuvent être aisement analysés Le programme être implementé sur un petit ordinateur et son u comporte pas de conaissance techniques spécifi part de l operateur Dans cet article sont des structure générale du programme et de ses sous-p principaux et sont rapportés des examples d'a tracés ENG

ZUSAMMENFASSUNG

Es wurde ein interaktives Programm fur die Anal vestibularen Nystagmus entwickelt Diese r Struktur des Programms gibt den Ärzten die M

: dunkelsten Teile des Nystagmogramms zu ieren (Bestimmung des Anfangs und des Endes wort des Nystagmus oder des Augenblicks der ung des Nystagmus, Beseitigung der Fehler) nen auch die unregelmassigsten und kompli- Linien, die man gewöhnlich in der klinischen antrifft, leicht analysiert werden Fur dieses im braucht man nur einen kleinen Allzweck er, der keine besondere technische Kenntnisse erateur verlangt In dieser Arbeit werden die ne Struktur des Programms und seine wichtigsten me beschrieben Es werden auch Beispiele der von ENG angefuhrt

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E. Mira, M D
Otorhinolaryngological Clinic
University of Pavia
27100 Pavia
Italy

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LOCAL TISSUE EFFECTS OF SURFACE-APPLIED ENT DRUGS

P-I Brånemark, O Hallen, K Nilsson, J Lindström and J Brunell

From the Laboratory of Experimental Biology, Department of Anatomy and the Department of Ot
University of Goteborg, Goteborg, Sweden

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Abstract Local tissue effects caused by a selected group of topically applied ENT-drugs are analysed By means of a testing system consisting of vital microscopy, infrared thermography and microangiography applied to hamsters and rabbits, an evaluation of the varying degree of tissue injury is made According to the degree of tissue damage the tested substances could be graded in three groups (I-III) The drugs belonging to group I show fairly little microcirculatory disturbance, while those belonging to group III cause tissue necrosis Our conclusion is that topically applied drugs should be used with caution, especially on previously injured tissues

Drugs which are applied onto or into tissues may damage normal tissue as well as diseased tissue, as demonstrated in studies on local tissue effects of wound disinfectants, corticosteroids, sodium, fluorides, chymopapain, and X-ray contrast media (Brånemark, 1967, 1969, 1972, Sörensen & Nilsson, 1972)

This injury is more pronounced in tissue where the covering epithelial barrier has disintegrated or the tissue itself has a lowered threshold for injury, as in inflammatory conditions Tissue injury is mainly mediated and manifested by the microvascular system

For the analysis of the response of the microvascular system to topically applied drugs, an experimental system was designed It consists of *vital microscopic evaluation* of drugs applied onto intact epithelium, onto experimentally denuded subepithelial structures, and injected into epithelium-covered tissue in the hamster's cheek pouch This model enables analysis of tissue microcirculation over 6-8 hours of the drugs as well as the restitution of the microvascular system can be The long-term consequences for the ti drug are delineated by *microangiograp* of microvascular reaction to the dr subperichondrally in the rabbit's dynamic events in the area of the ear drug was deposited, can be evaluated *thermography* Macrophotographic of the ear in transillumination give ditional information on the inflamm tion

The purpose of the present investi to elucidate the possible tissue injury by ENT drugs which are clinically a mucous membranes with intact or disintegrated epithelial covering

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MATERIAL AND METHO

The following drugs were analysed *selected as representing some commo* topically applied substances being use practice It may be observed that in n drugs is the pharmacological effect be corrosive

1 Carbocain Thesat 4+1 %
2 Carbocain Thesat spray 5+2 %
3 Chloromycetin Topical
4 Kenacort
5 Mercuro
6 Nafazolin



Bird s eye view of exposed cheek pouch of hamster

ebocetin	12	Terracortril Polymyxin B
ezeril	13	Tetracain 1 %
trivin	14	Tetracain 2 %
octanin	15	Xylocain 4 %
irgesin	16.	Xylocain spray 10 %

al microscopy of hamster cheek pouch

d and material Hamsters of both sexes ng about 100 g were anaesthetized with utal 30 mg/kg i m The animal was ilized in a special microscope stage with dy temperature maintained The pouch posed over a glass plate so that a proper nsor system could be used for trans-nation (Fig 1) The cheek pouch was uously irrigated with Tyrode s solution nstant temperature This is important as rculation in the cheek pouch is sensitive ydration and changes in temperature

ree experimental designs were used (1) epithelium, (2) production of a defect ion of tissue), and (3) deposit

Each substance was applied onto intact lium once in every experiment

In 30 experiments the pouch was everted he epithelium and connective tissue on one over an area approximately 2 mm in diameter, were gently removed by means of a stereomicroscope and microsurgical instruments (Fig 2) This procedure results in a microwound with exposure of connective tissue capillaries in the defect The medium was applied with an eye dropper in such an amount that maximal effect of the drug was established during the application, after 10 minutes the medium was washed away with Tyrode's solution

(3) In 64 experiments 0 05 ml of the drug was injected into the upper layer of the cheek pouch (Fig 2), the pouch then being watched for up to 5 hours under standard environmental conditions maintained by irrigation with Tyrode's solution The observations were made with transillumination in a modified Leitz intravital microscope (objective UO X 23, NA 0 55 and UO X 55, NA 0 84) with microphotographic recording of structures and microcinematographic registration of flow patterns (Brånemark, 1964)

Corpuscular flow velocity was measured in selected small venules by a comparative optic-electronic method (Brånemark & Jonsson, 1963)

B *Infrared thermography (IRT)*

Twenty four rabbits of both sexes weighing approximately 1 5–2 kg were used for repeated thermographic studies of the heat emission pattern from the ears Heat emission from rabbits ears predominantly emanates from the subcutaneous vessels Increased or decreased circula tion is reflected as a change of the surface temperature

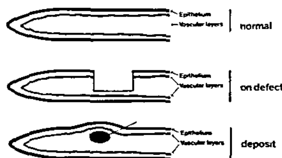


Fig 2 Diagrammatic representation of various experimental procedures for vital microscopic evaluation of microcirculation in hamster cheek pouch

The IRT registrations were performed with an AGA Thermovision mod 669 The instrument was calibrated against a temperature reference The spatial resolution capacity for the actual experimental set-up allowed of separation of individual vessels with a diameter of around 0 5 mm The thermal resolution was about 0 4°C The room temperature was kept within 22 ± 1°C The heat emission from each ear was registered before the deposition of the substances Registrations were then performed immediately after deposition and thereafter once a day during one week

Three parameters were used for evaluation of the local toxic effects of the tested substances

1 Immediate decrease of heat emission from deposit area This parameter indicates the vasoconstrictive capacity of the deposed substance and does not necessarily parallel the definite tissue damage

2 Development of emission pattern *in the deposit area* as an indication of inflammatory and healing reactions or grave tissue injury with irreversibly blocked circulation

3 Development of emission pattern from *marginal areas, not in direct contact* with the deposited substance

Each of these parameters could be graded in relation to the time factor and to the extent of the temperature increase or decrease as well as to the area involved

Microangiography

he same 24 rabbits as used in section B, were also subjected to microangiography The ears were gently shaved and 0 1 ml of the solution to be examined was injected into the subperichondrial tissue with a 16-gauge needle Each medium was cross deposited proximally in one ear, distally in the other ear

The state of the ears was clinically evaluated and photographically recorded in transillumination for 7 subsequent days The ears were also used for thermographic analysis during the same period

One week after deposition the rabbits were anaesthetized with approx 100 mg of Nembutal® intravenously and microangiography was performed The thighs were shaved and the rabbit was positioned on its back An incision was made through the inner surface of the thigh to expose the femoral artery This was cannulated with a fine polyethylene catheter Approx 2 ml of a 1 1 mixture of heparin (2%) and 1/2% Xylocaine was introduced via the catheter A 25% suspension of carefully agitated Micropaque® (1 part barium sulphate to 4 parts physiological saline filtered through a Pyrex filter of porosity 1) was then injected into the catheter at a controlled constant pressure of about 130 cm of water, which was just above the arterial pressure of the rabbit Both jugular veins were divided and the rabbit allowed to slowly exsanguinate over a period of 30 to 40 minutes as the Micropaque gradually filled the intravascular space During this time 4 more ml of the heparin xylocaine mixture were introduced in divided doses The infusion of Micropaque was continued about 30 minutes after the death of the rabbit An average of ... ml of Micropaque was introduced and a mixture of blood and Micropaque were collected from the cut jugular veins The ears were then clamped at their bases with large compression forcepses and severed from the head proximal to the forceps

The ears were placed outer side down on Kodak MR X ray plates which were covered with a thin aluminium foil They were exposed to X-rays from a Machlett ... X-ray tube at a distance of 120 mm set at ... and 15 mg for 15 minutes

RESULTS

Vital microscopy, hamster cheek pouch

In the test situation with intact epithelium only drugs which in the other test situations showed severe damage caused recognizable microcirculatory disturbances such as ... and microemboli

In the other two experimental situations the microvascular disturbances caused by different drugs could be differentiated ...




F (*a* *b*) Hamster cheek pouch Vital photomicro- f venular system (*a*) before, and (*b*) after applica a drug belonging to group I causing slight microvascular injury with some venular dilatation in (*b*) and increased hematocrit due to temporary slow-down of flow

groups on basis of the severity of the dam- the microcirculation In the first group fairly moderate microvascular interference was a slow down of the flow mainly in the s immediately after application of the Occasional occurrence of spherocytes ted hypo-osmolarity Some white cells rolling alongside the venule walls and rary standstill occurred in a few vessels hanges were of a transient character and vascular function could be restored A dilatation in the microvascular compart occurred (Fig 3*a*, *b*)

the second group the microvascular dis- nce was more pronounced, with slow-down ow in most of the microvessels and pro- ced osmolar disturbances However, when rug was removed by irrigation with saline, was regained in most of the vessels There a distinct phase of hyperemia White cells were rolling alongside the endothelium, microthrombi and microemboli were characteristic features Thus, the microvascular function was not completely restored and there were definite signs of microvascular injury (Fig 4*a*, *b*, *c*, *d*)

In the third group a severe injury to the microcirculation could be registered with immediate and complete blockage of flow and intravascular hemolysis There was no restitution of microvascular function in this group (Fig 5*a*, *b*)

A clear dose-response situation is exemplified by tetracain which in 1% solution showed changes placing it into group II, but in 2% solution, to group III

Macrophotographic registrations

Obvious classical inflammatory reaction around the deposit area could be registered for the substances causing severe tissue damage In a few cases the central part of the deposit area was

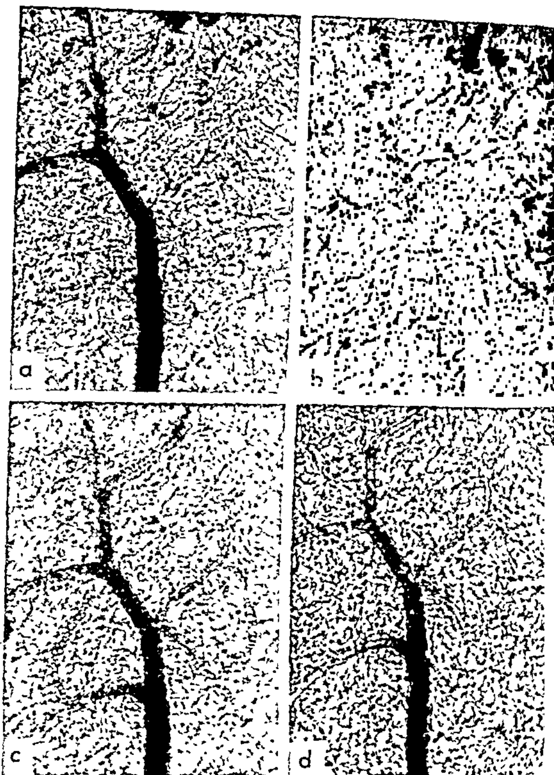


Fig 4 (*a*, *b*, *c*, *d*) Hamster cheek pouch Vital photomicrogram of venular system (*a*) before, and (*b*) after application of a drug belonging to group II (*c*) Early restitution phase after irrigation with sa spherocytes in postcapillary venules. (*d*) R phase with dilated microvessels and high b





(a b) Hamster cheek pouch. Vital photomicro- ... of venular system (a) before and (b) after applica... a drug belonging to group III causing complete blockage of the microcirculation and permanent tissue damage There was no restitution of flow after irrigation of saline

...c at the end of the investigation The ...pment of oedema and hemolysis often ...a more detailed judgement of the under ...tissue condition impossible

...ngiography

...microangiographic evaluation of micro-...ar reaction to subperiochondrally de...d drugs was performed after 7 days The ...ons were characterized either by slight microvascular proliferation, marked micro vascular proliferation, or tissue loss and border ing vessel proliferation respectively These changes could be used to separate the drugs into three main groups, the first of which thus shows only minor microvascular damage, the second of which initiates definite damage of a proliferative character and the third of which causes loss of tissue and heavy marginal vessel proliferation (Fig 6*a*, *b*, *c*)

...I *Microvascular injury caused by topically applied ENT drugs*

...I	Group II Definite but reversible	Group III Severe non reversible
...tin	Nafazolin	Carbocain Thesat 4 1 %
	Tetracain 1 %	Carbocain Thesat spray 5 2 %
		Chloromycetin Topical
...in		Kenacort
...ortr l Polymyxin B		Mercurocrom
...un 4		Pyoctanin
		Tetracain 2 %
		Xylocain spray 10 %







Fig 6 (a–c) Microangiograms of rabbit ear demonstrating in (*a*) slight microvascular proliferation caused by a drug belonging to group I in (*b*) some marginal vascular proliferation around central area of damaged tissue caused by a drug belonging to group II (*c*) Tissue necrosis surrounded by proliferating microvessels after application of a drug belonging to group III

Infrared thermography

A stepwise reaction to the deposi could be registered For almost al an initial decrease in heat em a deposit area could be seen Th s turned to normal within one day fo of substances without any sign of marginal emission indicating infl action

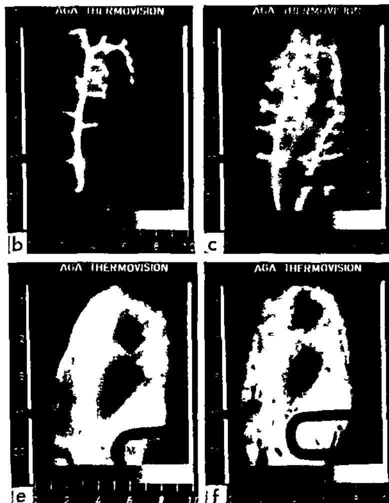
In another group the central returned to normal within 3–4 da parallel marginal increase of tem the deposit area and an increase of emission from areas outside the dep For some of the substances the cen area did not return to normal registration period The marginal t increase was vigorous and the em other parts of the ear strongly incre 7a f)

According to changes in heat em drugs could be separated into three were identical with the grading ach vital microscopic studies

Thus the information gained from experimental approaches were entirely and could be summarized according to

COMMENTS

This study of local tissue effects of applied ENT drugs has been perf hamster s cheek pouch and rabbit s ea parameters have been considered rel *immediate effects* on the flow of microvascular compartments The *consequences* of the microvascular dis have also been evaluated On the bas present investigation and on our prev of other drugs using a similar exp set up it appears justified to conclude the point of view of the integrity of of its restorative capacity and with both normal and diseased tissue drugs to group III in our grading system used with great caution This comm with full appreciation of the fact th



f) Infrared thermograms of rabbit ear visualizing in surface temperature (*a*) and (*d*) represent the before deposition of drugs belonging to group II respectively (*b*) and (*e*) show emission patterns days (*c*) and (*f*) after 7 days Note minor changes in sequence *a–c* in contrast to strong and irreversible changes in sequence *d–f* with marked central decrease of heat emission surrounded by patchy and vigorous increase of heat emission indicating inflammatory reaction.

from animal experiments cannot be iately transferred to clinical practice and Drugs belonging to group II should be with caution especially in cases where the s applied onto a tissue which is already l Drugs belonging to group I cause fairly issue damage, if there is no previous injury tissue If however there are already vascular disturbances in a tissue even this additional injury can be an important influencing the healing capacity of the

Summing up then, this experimental analysis of topically applied ENT drugs has shown that some of the drugs used in clinical routine cause tissue necrosis, while others show distinct but reversible microvascular damage, whereas some drugs cause fairly little microcirculatory disturbance

The methods used have proved to be sensitive enough to register even discrete tissue impairment at the microscopic level It can be recommended that surface applied drugs as well as the vehicles involved be tested in this respect

ZUSAMMENFASSUNG

Die Gewebeschäden einer Gruppe ausgewahlter, lokal applizierter Arzneien, die in der HNO Praxis verwendet werden, wurden analysiert Die Analyse wurde mit Hilfe eines Testsystems, bestehend aus Vitalmikroskopie, Infrarot Thermographie und Mikroangiographie, durchgefuhrt Durch Applizierung der Medikamente an Hamster und Kaninchen wurde eine Bewertung der Gewebeschäden erreicht Dem Grad der Gewebeschäden entsprechend sind die getesteten Substanzen in drei Gruppen eingeteilt worden Arzneien der Gruppe I verursachen nur sehr kleine mikrozirkulatorische Storungen, demgegenuber verursachen die der Gruppe III Gewebenekrosen Das Endresultat unserer Untersuchung zeigt, dass lokalapplizierte Arzneien stets mit grosster Vorsicht angewandt werden mussen besonders wenn es sich um beschädigtes Gewebe handelt

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O Hallén, M D
Dept of Otolaryngology
University of Göteborg,
S-413 45 Goteborg
Sweden

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ULTRASTRUCTURAL FEATURES OF HUMAN JUVENILE LARYNGEAL PAPILLOMAS

P-G Lundquist, L Frithiof and J Wersäll

From the Department of Otolaryngology and King Gustaf V Research Institute, Karolinska Sjukhuset, and the Department of Oral Surgery, School of Dentistry, Karolinska Institutet, Stockholm, Sweden

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t The ultrastructural organization of 12 juvenile al papillomas was investigated Four patients were ıan 40 years old but had carried the disease since od The other 8 patients were 2 10 years old and ried the disease from 1–5 years. There is regularly d a discontinuity of the basement membrane and al cells often exhibit abundant cytoplasmic pro- In the stratum spinosum there is often found n like inclusions as well as Odland bodies and ıyalin granules In the superficial layers there are remnants as in parakeratosis Virus like particles meter around 330 Å have been demonstrated in a rated superficial cell The scarcity of such particles ıssed with possible regard to a transformation ıg in the superficial layers of the papilloma and ıigh shedding rate of the surface cells

ıman laryngeal papilloma of the juvenile a benign epithelial tumour at the anterior f the vocal cords and which has a tendency ead along the epithelium, often involving he ary-region as well as the ventricular

enile papillomas tend to recur after exci- in some cases with intervals of one or ıl months Permanent cure is often not ved until adolescence and even then, per ; hoarseness of varying degree is not un- ıon There is reason to believe that the gy is viral but there is so far no convincing ific evidence

pillomas of the adult type are far less apt cur after excision The etiology is con- ed to differ from that of the juvenile A ivable viral etiology is not supported by cientific results, but cannot be excluded

The purpose of the present study is to analyse the ultrastructural features of juvenile laryngeal papillomas and to discuss the possible pathological significance

MATERIAL AND METHODS

The material consists of biopsies from one normal and twelve pathological vocal cords of the human larynx The specimens were excised under general anaesthesia at the Department of Otolaryngology, Karolinska sjukhuset The pathological material includes eight papillomas from children, and four papillomas from adults (Table I) The latter group, however, can also be regarded as belonging to the juvenile type as they have persisted since childhood (Table I)

Excised tissue was divided in two parts, one for histopathological diagnosis and one for electron microscopy Specimens for light microscopy were fixed in a 10% solution of formaldehyde Specimens for electron microscopy were fixed in a buffered 1% solution of osmium tetroxide according to Rhodin (1954) Specimens for electron microscopy were dehydrated in ethanol and embedded in Epon They were sectioned with an Ultrotome (LKB) Thin sections were collected on Formvar coated copper grids and stained with 1.5% uranyl acetate solution (Watson, 1958) and with lead citrate (Reynolds, 1963) Observations were made in a Siemens Elmiskop I operated at 80 kV

Table I *Juvenile laryngeal papillomas investigated*

Age at biopsy (years)	Age at onset of disease	Sex		Total number
		Male	Female	
2–10	2–5	5	3	8
>40	<8	2	2	4

OBSERVATIONS

Normal material

The basement membrane region The subepithelial connective tissue is loose in structure with vessels, cellular elements and scattered collagen fibrils (Fig 1) The connective tissue is separated from the epithelium by a continuous basement membrane about 600 Å thick (Fig 1)

Stratum basale The basal surface of the cuboidal basal cells is comparatively smooth due to the absence of cytoplasmic processes which appear in great number on cellular surfaces facing adjacent basal or spinous cells The cytoplasm (Fig 1) is structurally dominated by the high concentration of dense bundles of tonofilaments, some of which seem to be closely related to desmosomes and half-desmosomes In the cytoplasm there are also numerous round or ovoid mitochondria of high electron density, ribosomes interspaced between the tonofilament bundles, and a few vacuoles of varying dimensions In the supra nuclear region, numerous all vesicles may be seen forming the Golgi tem The nucleus of the basal cells (Fig 1) large, forming an irregular sphere with wide as well as narrow indentations, furrows and clefts The nucleoli are well developed and the main part of the dense chromatin appears at the nuclear margin Intercellular spaces are narrow with numerous slender interdigitating cytoplasmic processes (Fig 2) The intercellular space is bridged by wide, tonofilament-containing processes from opposing cells joined by desmosomes In the basal cell layer the desmosomes are sparse White blood cells appear in the intercellular space They are not attached to the epithelial cells by desmosomes (Figs 1 and 2).

Stratum spinosum In this layer the general structural features as described above r tained However, the number of cyto cesses is increased, compared with the layer Processes of the slender as wel wide types are numerous and there ar desmosomes present

In the superficial part of the stratum the cells and their nuclei gradually a flattened shape Numerous Odland bod land, 1960) are seen close to the plasm brane (Fig 3) Keratohyalin granules se absent. Vacuoles with a moderately de tent appear in the cytoplasm (Fig 4).

Stratum superficiale In these cells the nuclear chromatin is increase sity and the nuclear membrane is indist in the surface cells the nuclear remnants the cytoplasmic structures are identifie

Pathologic material

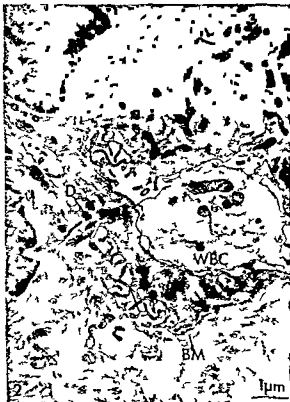
General features In all specimens thelium is of the stratified squamous papillomas the stratification is far le than in the normal tissue as the size

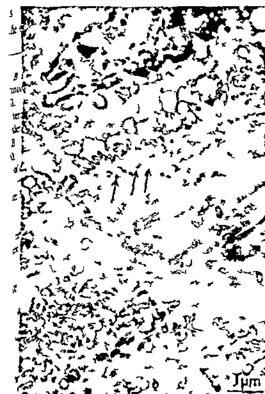
Fig 1 Normal larynx The basal cells (*B*) ar from the connective tissue (*Ct*) by a continuo membrane (*BM*) *WBC* White blood cell ×
Fig 2 Normal larynx In the narrow interce between basal cells there are numerous slende (*arrow*) Desmosomes (*D*) are few *WBC* W cell *BM* Basement membrane ×9 000
Fig 3 Normal larynx Superficial part of stra sum There are numerous Odland bodies (*arrow*
Fig 4 Normal larynx Stratum spinosum (*S*) superficiale upper part of picture *I* Va nuclear remnants ×9 000
Fig 5 Laryngeal papilloma Basement membr Cytoplasmic material (*Cy*) is spread into the

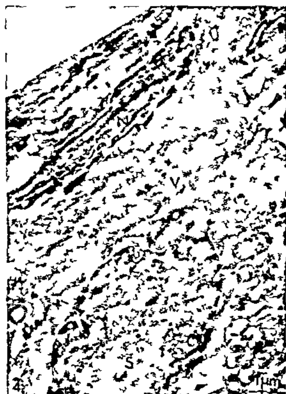
tive tissue ×3 000
Fig 7 Laryngeal papilloma stratum basale

plasm of the basal cells is almost complet mitochondria (*Mi*) *Ct* Connective tissue ×5 000


BM
Cl

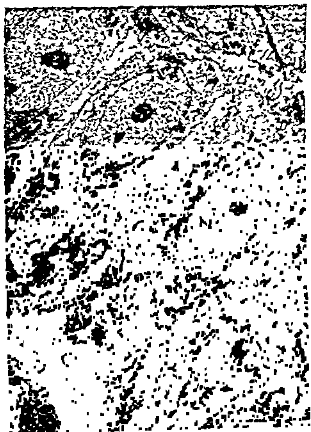

D
WBC
BM
2
1μm


1μm


N
V
S
4
1μm




6




N
C
8



9 Laryngeal papilloma, lower spinosum Heavy its of glycogen like material (G) ×53 000 (b) Appearance of the glycogen like material (G) at lower magnification ×5 300

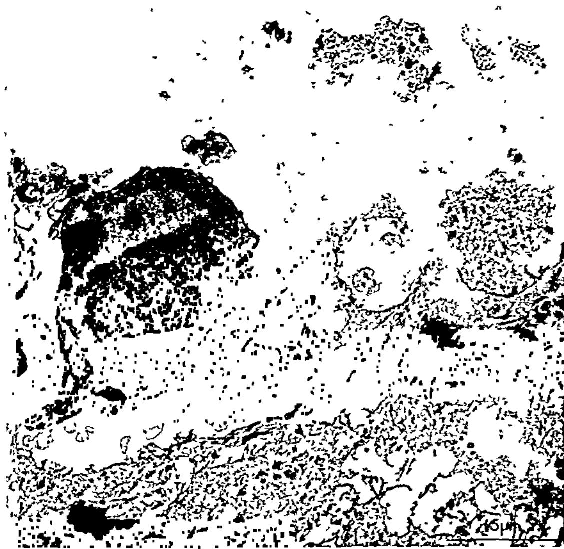


Fig 10 Laryngeal papilloma, stratum spinosum. A con- arrangement of cells is occasionally seen. × 3 000

shape as well as the orientation of the cells vary. White blood cells present in the epithelium contribute to the irregularity of the pattern The size of the cells seems to vary more than in the normal tissue Cells smaller than normal are far more numerous than enlarged cells The variability of the cytoplasmic and nuclear structure is markedly increased, compared with the normal tissue.

The basement membrane region The structural composition of the basement membrane is normal It is frequently discontinuous, however, and cytoplasmic processes basal cell extend into the connective one specimen, cytoplasmic material the connective tissue in an area where ment membrane is absent and the plas brane of the basal cell is broken (Fig basal lamina is frequently duplicated as the irregular basal surface of the ba Numerous white blood cells in places the subepithelial area close to the basal

Stratum basale The basal cells d ing structural features in different specim



1 Laryngeal papilloma Atypical chromatin pattern in stratum spinosum. × 6 000

lifferent areas Some of them are highly ron-dense with a high concentration of tono-nents and ribosomes (Fig 6) and exhibit ndant cytoplasmic processes The intercellular ce is markedly increased in width in most imens Long and slender cytoplasmic pro-es extend from the basal cell Wide-based, mosome-associated processes are rare (Fig In other basal cells the cytoplasm appears to almost completely filled with mitochondria g 8) In two specimens heavy deposits of cogen-like material appeared in the basal cells (Fig 9) The nuclei are ovoid in shape and, compared with the normal specimen, most of them appear swollen, with only traces of chromatin attached to the smooth nuclear margin (Figs 7 and 8)

Stratum spinosum Usually, the general types of structural patterns identified in the basal cell layer are maintained in the lower part of stratum spinosum

Abundance of mitochondria and glycogen-like material, wide intercellular spaces and the changed nuclear structure are features which,



when present in the basal layer, also appear in the lower spinous strata In the central and superficial parts of the stratum spinosum some changes appear In some specimens, spinous cells are seen concentrically arranged around a dense cluster of a few cells, thus distorting the regularity of the stratification (Fig 10)

There are also cells with considerable deposits of chromatin arranged along the nuclear border and with atypically deposited chromatin (Fig 11) In the superficial part of the stratum spinosum, Odland bodies appear and the cells ally tend to assume a flattened shape

In one specimen several cells were fou a filamentous nuclear material leaking cytoplasm (Fig 12)

Stratum superficiale The flattened the stratum superficiale are usually close ranged but in some areas the wide inter space is maintained throughout the epi

The cytoplasmic organelles are identified in the surface cells There are cytoplasmic



Laryngeal papilloma In the dense cytoplasm of al cells, the organelles are still present There is in pattern ×5 000

vesicles and nuclear changes with loss of r structures, increased density of the chro- and disrupted nuclear membranes (Fig one previously mentioned specimen (Fig 'akage" of nuclear material into the cyto- was seen also in several superficial cells 4) These cells did not, however, display the lic changes usually seen associated with r rupture Occasional round keratohyalin es are seen (Fig 15) In several disintegrat- clei in superficial cells there are round dense les varying in diameter from 200–500 Å

In one disintegrated superficial cell of a papilloma, numerous particles about 330 Å in size were seen (Fig 15) Nuclear remnants are recognized even in the surface cells which are parakeratotic or nonkeratinized The size distribution of the particles is seen in Fig 16

DISCUSSION

Normal material

The general ultrastructural features of the normal material are in agreement with the structure of the normal parakeratotic epithelium which



Fig 14 Same specimen as in Fig 12 Stratum superficiale Filamentous (*f*) nuclear material extends into the cytoplasm *Nm* Nuclear membrane ×25 000

may be seen in certain parts of the oral cavity (Frithiof, 1969) The continuous and regular basement membrane, the abundance of tonofilaments and ribosomes in the basal cells contribute to this pattern In the higher strata, numerous Odland bodies (Odland, 1960) are found An obvious scarcity of keratohyalin material is a further characteristic The presence of nuclear remnants and cytoplasmic organelles in the condensed cytoplasm of the surface cells is further evidence of the parakeratotic nature of the epithelium.

Pathologic material

In the laryngeal papilloma the epith keratotic. There are structural ch basement membrane region Similar known to occur in a number of path tions involving epithelial tissues (T Eriksson, 1972) The structural vari is observed in all cell layers, is ind pathological process The abundan chondria and heavy deposits of glyc metabolic disturbances The nuclear cluding atypically deposited chroma



[illegible]15 Laryngeal pap lloma Part of a d s ntegra ed [illegible]s (N) wi h virus l ke particles (V) Kh Kerato- 77 000 (a) The nuclear and cytoplasmic d s ntegrat on s typ cal of a pap lloma v rus nfected cell 5 000

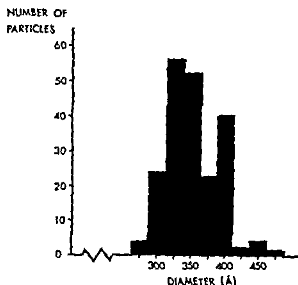


Fig 16 Size distribution of 200 virus like particles

age of filamentous nuclear material into the cytoplasm might be of interest when the possible viral etiology of the disease is discussed

It is generally accepted that papilloma (wart) viruses, belonging to the papovavirus group (Melnick, 1962) are the etiological agents in animal papillomas and human warts (De Monbreun & Goodpasture 1932, Shope, 1933) The ultrastructure of viral particles in human warts has been demonstrated by several authors (Almeida et al, 1962, Bunting 1953, Strauss et al, 1949, Williams et al, 1961) Similar virus like particles ere found in superficial cells of human oral pillomas (Frithiof & Wersall, 1967) It has been observed that the papilloma virus contain-

Table II *Diameter of papilloma virus like particles in sectioned tissue*

Author	Year	Particle diameter (Å)	Host and site of lesion
Bunting	1953	290	Human skin
Stone et al	1959	330	Rabbit skin
Charles	1960	330	Human skin
Williams et al	1961	340	Human skin
Rdzok et al	1966	380	Rabbit, oral cavity
Frithiof & Wersäll	1967	350	Human oral cavity
Present observation		330	Human larynx

ing cells have a specific structure (Ch... Cheville & Olson, 1964)

In spite of a thorough search for ... particles in all 13 specimens only one superficial cell in one specimen reve... pattern characteristic of virus infected c... an aggregate of virus-like particles 330 Å ... meter The size is in agreement with viru... les found in thin sections of warts and p... from human and rabbit skin and oral (Table II) The shape and structure particles closely resemble the structure of ...loma virus in sectioned tissue As s... confirmatory virological experiments ha... been performed, the particles describe should be referred to as "papilloma v..." on purely morphological grounds

Previous reports on human laryngeal ...loma have not revealed any papilloma v... particles Attention has been focused c... plasmic particles with a diameter ab... times that of the papilloma viruses (Me... Schulz 1957, Timmel, 1961)

It is possible to consider the papillom... as an etiological agent of laryngeal pap... spite of the fact that virus like particles ... in only one cell in one specimen Most ... the viruses exert their influence on ... germinative layer (Cheville & Olson ... Shope, 1962, Stone et al, 1959) It h... repeatedly stated in the literature that t... like particles are not seen in the basal c... only in the spinous and superficial cells (1962) deduced that animal tumour vir... cluding papilloma virus, are not alway... like, and that investigations should be e... to include a search for nonvirus like age...

From the present study it is obvious t... extremely difficult to find virus like part... laryngeal papillomas The reason may b... the transformation of the unrecognizable ... presumably present in the basal and s... cells, to the recognizable virus like p... occurs only rarely in laryngeal papilloma... to a high shedding rate compared with th... mon wart in which virus like particles are ... demonstrated

suggested that the transformation of the › into a recognizable shape occurs as a of a decreased or changed metabolism of perficial cells Due to the high rate of protion and shedding of the surface cells, the mics of viral transformation will render the ce of recognizable virus-like particles a in juvenile laryngeal papillomas

ZUSAMMENFASSUNG

trastrukturelle Organisation von 12 juvenilen papillomen wurde untersucht Vier Patienten waren Jahre alt und hatten seit ihrer Kindheit an der eit gelitten Die ubrigen acht Patienten waren im on 2–10 Jahren und hatten die Krankheit seit 1–5 Regelmassig wurde eine Diskontinuitat der Basalan beobachtet, und die Basalzellen entwickelten zahlreiche zytoplasmatische Prozesse Im Str m wurden oft glykogenartige Einschlusse, Od orper und Keratohyalin-Granula gefunden In den hlichen Schichten fanden sich Kernreste wie bei atose Virusähnliche Partikel mit einem Durchvon ca 330 Å wurden in einer desintegrierten chenzelle festgestellt Das seltene Vorkommen Partikel wird diskutiert, und dabei wird auf eine ie Transformation in den oberflachlichen Schichten illoms und auf die hohe Abschilferungsrate der chenzellen hingewiesen

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P-G Lundquist, M D
Dept of Otolaryngology
Karolinska Sjukhuset
S 104 01 Stockholm
Sweden

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EXPERIMENTAL STUDIES ON THE TREATMENT OF UNILATERAL VOCAL CORD PARALYSIS

M Neiger and R Baumann

From the Department of Otolaryngology, University of Bern Bern, Switzerland

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[illegible] surrounded by conjective tissue whereas the Silicon is enclosed in an unic membrane We could not see a migra tion of the materials in the depth

The injection of Teflon behind a paralysed vocal cord is a well known prognostic and therapeutic method The reaction of the surrounding tissue to the foreign body has previously been described by Arnold (1962), Reichhardt (1970), Goff (1973) and others

Besides Teflon we have made an experimental study of another material—Silicon Both materials were tested in animals living under normal tions We wished to check whether parts of the foreign body migrate or are absorbed. Teflon was extremely favorable for this study, as its particles are birefringent and therefore easy to localise under polarised light In three 12 week old lambs we injected Teflon paste behind one and Silicon liquid behind the other vocal cord Silicon was sterilised immediately before the operation at a temperature of 120°C for 10 minutes Temperature and length of sterilisation determine the viscosity of the Silicon liquid

All the animals endured the operation well they did not seem to have any trouble and developed normally afterwards One of the sheep gave birth young after a normal pregnancy

After 3, 6 and 10 months the animals were killed, the larynx was removed i frozen in liquid nitrogen Frozen s 30 μm were made and stained with i eosin Whereas the sectioning of injected side raised no problems t of the Silicon injected side was dif Silicon was expelled during the pro

We found two types of tissue r the Teflon paste (1) The Teflon lay deposit encapsulated by a dense fi and completely separated from the tissue (Fig 1) Outside the capsul particles were visible (Fig 2) This tion definitely explains the observat in the cheek of a hamster, inasmuch localisation of the Teflon deposit changed. Once walled in by the fi the foreign body can no longer m foreign body could lie as a deposi neither enclosed by a dense capsule tely separated from the surrounding 3) as demonstrated by the muscle fi through the Teflon particles (Fig 4)

These two forms were not related t of time that the Teflon remained in we found the first form in the anim 3 and at 10 months, the second in killed at 6 months It seems far mor the site of injection, or the surround decisive Thus, we found the first fo tion when Teflon was injected in in fatty tissue, *the second when*

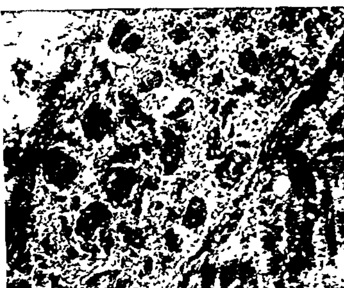


Fig 1 Teflon deposit with strong connective capsule



Fig 2 No Teflon particles visible outside the capsule under polarised light.



Fig 3 Teflon deposit without capsule in between muscle tissue.

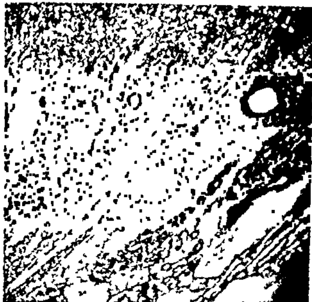


Fig 4 Muscle fibres (*arrow*) in Teflon deposit



Fig 6 Infiltration of histiocytes and lymp

tissue such as muscles or glands were present Whereas the Teflon pushed aside the tissue in the first case, it *infiltrated existing cleavage spaces in the second case*

The cytological reaction remained the same in both previously described types The *Teflon particles induced a strong conjunctive reaction,* inasmuch as they were surrounded by the connective tissue (Fig 5) Furthermore, we noted a *moderate infiltration of histiocytes an* cytes (Fig 6) and *more or less gia* foreign body type (Fig 7)

As concerns the migration of t particles, some of them were foun distance from the large deposits (Fig *studying their localisation in serial* was obvious that they either rema canal of injection, or that they bel *more widely spread Teflon deposit,* as

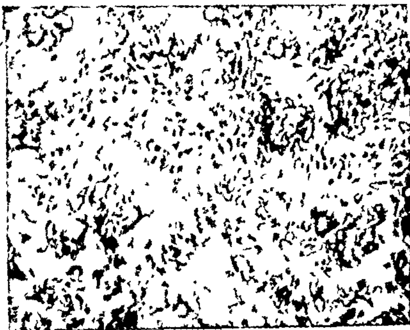


Fig 5 Connective reactio

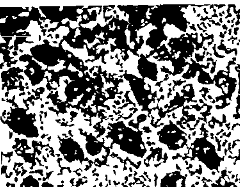
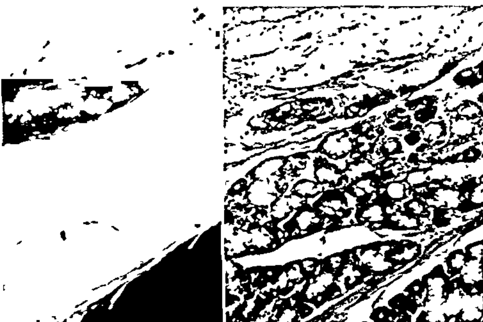


Fig 7 Giant cells of foreign body type



ʾarticles of Teflon in the canal of injection

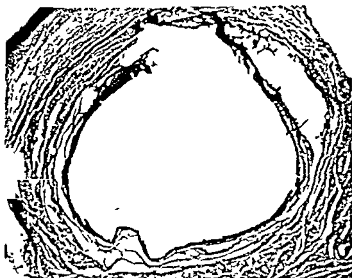


Fig 9 Pseudo-cyst caused by Silicon injection

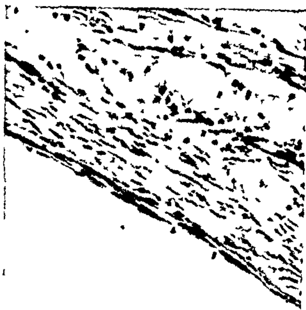


Fig 10 Border of the Sil con pseudo-cyst

ın form two It was not possible to detect Teflon particles further away from the deposit nor in lymphatic spaces It is therefore most probably that no migration of Teflon takes place

As to the Silicon, we can only answer the question of reaction on the tissue Beside the sections of the larynxes we also studied some sections of ears of rabbits There was here a marked difference between Teflon and Silicon The connective tissue did not infiltrate the Silicon nor did any cells migrate into the foreign body The Silicon remained like a cyst in the tissue (Fig 9) The wall of the cyst showed a certain increase of connective tissue but without forming a real capsule A slight infiltration of histiocytes and lymphocytes was present The border of the tissue looked like a pseudo endothelium with flat and long spread cells (Fig 10)

We could not find any giant cells of [...] type

Silicon therefore seems to be bett[...] than Teflon, furthermore it may hav[...] tage of being removed more easily i[...] expected reactions Unfortunately it [...] been set free for use on human bein[...]

ZUSAMMENFASSUN[...]

Lammern wurde hinter das e ne Stimmba[...] hinter das andere Sil konpaste injiz ert [...] 10 Monaten wurden d e Kehlkopfe h st[...] sucht Dabei zeigte sich dass d e Teflonp[...] dichten bindegewebeartigen Kapsel eingesc[...] wenn sie im Fettgewebe lagen War d[...] Muskel oder Drusengewebe erfolgt leh[...] und das primäre Gewebe war zwischen den [...] noch erkennbar Silikon loste nur e ne k[...] aus und lag wie der Zysteninhalt in einer e[...] gewebeartigen Membran Ein Abwandern [...] tikel uber Lymphspalten konnte nicht fes[...]

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M Neiger M D
Dept of Otolaryngology
University of Bern
Inselspital
Bern
Schwitzerland

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HYPERPLASIA OF THE PAROTID GLAND U N S

A Stress Phenomenon?

S Ericson and A Wiberg

From the Departments of Oral Radiology and Otolaryngology, University Hospital Umeå Sweden

(Received April 4 1974)

...t The secretory function of the parotid glands as ...ated by the salivary secretion rate, has been ...d in 10 patients with parotid hyperplasia U N S ...nt levels of gustatory stimulation were used The ...n rate was reduced in 8 out of 10 of the patients ...derately high level of stimulation but normal at ...imum levels in all the 10 patients The results that the cause of the parotid hyperplasia may be ...ited sympathetic influence, possibly due to stress

...ement of the salivary glands is a common ... of various gland disorders such as sial-...is tumours, obstruction to secretion, and ...s The etiology of sialosis is obscure and discussed, a typical feature is the involve-...of the parotid glands, with bilateral swell ...nd an asymptomatic and often episodic ... (Diamant, 1959, 1960, Rauch, 1959, ..., 1962) Apart from the increase in gland radiological examination is usually of help in the diagnosis, it may, however, ...le other clinical disorders with similar ...estations A characteristic of the histo-...l picture is non-inflammatory swelling of ...land cells (Seifert, 1964, Pohto, 1966) ...ptomatic salivary gland hyperplasia, re-...l to as sialosis, has been observed in meta-... and endocrine disorders, liver complaints, ...ses, as a drug reaction, and nutritional defi-...y (Rothbell & Duggan, 1957, Rauch, 1959, ...rt, 1962, 1966, Borsanyi, 1962, 1963, Anger-...t al, 1963, Alappatt & Ananthachari, 1967, ...dson et al, 1969)

... has been induced experimentally in ... by adrenergic overstimulation with repeated injection of isoprenalin and other catecholamines (Selye et al, 1961, Seifert, 1964, Pohto, 1966, Ohlin, 1966) The resulting cell picture bears a resemblance to that of spontaneous sialosis in man, Seifert (1964, 1966) has accordingly advanced the hypothesis that a possibly important etiological factor of sialosis in man is an increase in the sympathetic tone followed by disturbance of the normal enzyme synthesis in the salivary glands It is not known, however, whether adrenergic stimulation has any causal connection with the sialosis observed in man The purpose of the present pilot study was to examine the response of the parotid glands to gustatory stimulation of various strengths in cases of parotid hyperplasia U N S in order to find whether the condition is accompanied by disturbances in the vegetative influence of the glands

MATERIAL AND METHOD

The study was performed on 10 patients—5 men and 5 women—ranging in age from 26 to 56 years, that were seen at the Department of Otolaryngology, University Hospital, Umeå, for hyperplasia of the parotid gland These patients constituted all the patients in whom a diagnosis of parotid gland hyperplasia U N S was made at the Department during the years 1965–72

All the patients were submitted to a general medical examination, which, besides the routine examinations, included liver function tests, sero-

Table I *The pathological picture in 10 patients with parotid gland hyperplasia U N S*

− No symptoms
+ Gland of moderate size moderate symptoms
+ + Large gland, marked symptoms

Case	Sex	Age	Durations of symptoms (years)	Enlargement of parotid		Xerostomia, subjective
				Right	Left	
1 KL	♂	41	1	+ +	+ +	−
2 AK	♂	56	3	+	+	−
3 GE	♂	41	½	+	+	+
4 RJ	♀	41	2	+	+ +	−
5 IH	♀	51	3	+	+ +	−
6 LC	♂	43	1	+ +	+ +	+
7 JE	♀	33	1	+	+	+
8 LE	♀	26	20	+	+	+
9 ES	♂	47	1	+	+	−
10 EJ	♀	41	4	+	+	−

logical analysis for the presence of collagenosis or sarcoidosis, tests for diabetes, a dietary history, and, in the case of the women, gynaecological examination There was no conclusive evidence of any other disease or hormonal dysfunction and a diagnosis of parotid gland hyperplasia U N S was recorded The duration of the disease and the symptoms are presented in Table I

In addition to the clinical examination the parotid glands were radiographed and their secretory capacity was determined The results were compared with those for a similar examination of 92 healthy control subjects, this material has been described elsewhere (Ericson, 1970, 1971) A sialometric examination of the parotid gland function was performed at relative rest and after oral stimulation with 1 and 6 % citric acid The gland function was judged to be normal or abnormal on the basis of a comparison with the values from the control group—both in absolute values and with account taken of the size of the glands

The sialographic examination of the parotid gland included a determination of gland size and registration of changes in or near the excretory ducts The gland size was represented by the area of the lateral projection of the gland on the sialogram This area has been found to be closely correlated with the true volume of the gland ($r = 0.95$, Ericson & Hedin, 1970) The results of the sialographic examin[ation] also compared with those for the cont[rol]

RESULTS

The swelling of the parotid glands w[as] with a duration of 3 to 4 weeks over [a] 6 months to 20 years Besides the swell[ing] sensation of tenderness there was sli[ght] tive dryness of the mouth in some of t[he] recording a low secretion on stimul[ation] the 1 % citric acid solution

The pattern of the salivary duc[ts] sialograms did not differ from th[at] healthy glands, apart from the gland[s] no radiolucent zones or areas w[ere] ramification of the ducts were obs[erved] were there deviations, destruction [or] alternations in the calibre of the [ducts] salivary calculus or strictures were [seen] By the superficial position and size of t[he] glands it was possible to see in all th[e] a marked lateral extension of the gla[nds] anterioposterior sialograms, this cor[responded] to the clinically observed swelling

The gland size, as represented by th[e] projection of the gland on the sialo[gram] presented in Table II All the glands (in one patient, case 8) were larger [than the] mean for the control group and in [all] patients the sizes uni and bilaterally

II *Area of projection of the parotid gland al sialograms in 10 patients with hyperplasia parotid gland U N S and the correlation ~e means for a control group of 92 healthy ıs*

–	Area on lateral sialogram, cm²			
	Right	Left	Total	Right–left difference
group				
	22.6	24 6	47 2	2 0
	21 8	21 4	43.2	0 4
	22.0	21 0	43 0	1 0
	21 1	22 9	44 0	1 8
	26 3	19 6	45 9	6 7
–	22 5	22 6	45 1	0 1
	18 8	16 7	35 5	2 1
	11 6	11 8	23 4	0 2
	24 9	23 8	48 7	1 1
	17 8	17 8	35 6	0 0
	20 9	20 2	41 0	1 5
	4 3	3 9	7 7	2 0
group				
	15 5	15 6	31 1	0 9
	2 7	2 9	5 4	0 9
ion analysis				
	3 25	2.88	3 15	0 78
ance	+ +	+	+	Not sign

e normal limits, 20 9 cm² (Ericson, 1970) difference in mean gland size in the hyper patients and the control group was cally significant, as represented by the ted area on the sialograms

order to find wether the parotid glands on side in a given patient were differently d by the disease the left–right difference alculated (Table II) In only one patient 5) was this difference greater than what e regarded as normal—namely a maximum 7 cm² (Ericson, 1972), this indicates a minantly similar development of the glands ch side

e secretion rate of the parotid gland on lation with 1 and 6 % citric acid is illus- l in Fig 1 There was a large individual bility however, in comparison with the hy controls, the secretion rate being on ige lower for the 1 % but higher for the 6 % citric acid The differences between the means were not significant, however For the 6 % solution the level of significance for the difference was $P < 0.10$ The secretion at rest, which was also recorded, displayed no difference from the control values

As the secretion rate is normally related to the gland size, the gland function for the 10 pairs was analysed with respect to this parameter The results are shown graphically in Fig 1, here the limits of the normal range—with a 95 % probability based on the control group—have been entered It is seen that on stimulation with the 1 % citric acid solution, 8 of the 10 pairs displayed a lower secretion rate than normal for the gland size, whereas with the 6 % solution the secretion rate was practically normal The 2 patients (cases 1 and 9) recording a normal secretion rate for the lower strength had the largest glands

DISCUSSION

In 7 of the 10 patients the size of the parotid glands as represented by the lateral projected area on sialograms was greater than is normally seen in healthy persons (Ericson 1973) Except for deviations in size the sialographic appearances of the gland did not differ from those of healthy glands Solely on the basis of the sialograms it is therefore impossible to distinguish the glands in the patients with parotid hyperplasia from normal but extremely large glands The remaining 3 patients with moderately large parotid glands did not show differences from the normal sialographic picture The analysis of the relationship between the secretion rate and gland size as represented by the projected area on lateral sialograms however, suggests that there were defects in the glands in 8 of the 10 patients In 2 of them (cases 1 and 9) it was impossible to decide whether the glands were affected by pathological alterations or whether they were only large in size, this being suddenly noticed by the patient Nor can an incorrect diagnosis be ruled out, since neither the clinical nor the radiological criteria for a

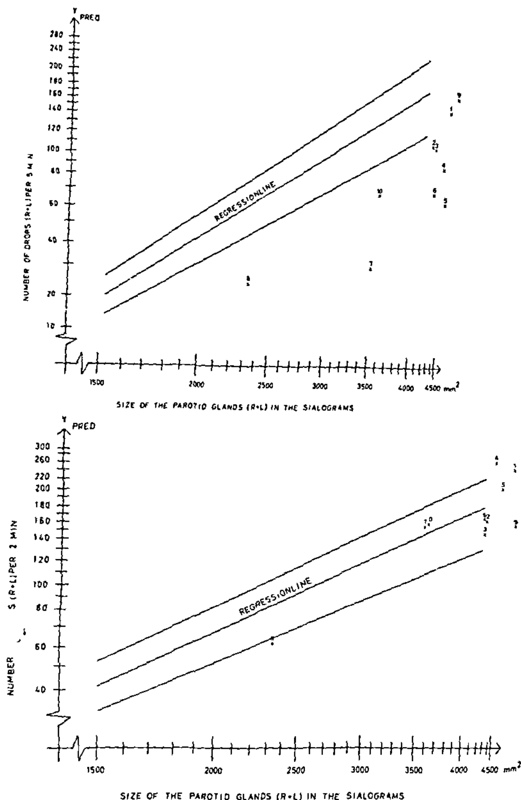


Fig 1 The rate of secretion of the parotid glands on stimulation with citric acid solution versus gland size, in 10 patients with asymptomatic gland enlargement The upper and lower lines represent the normal range for healthy glands (95 % confidence interval) The on the curves are case numbers *Upper graph* 1% acid solution *Lower graph* 6 % citric acid solution drop is equivalent to 0 06 g of saliva

sis of parotid hyperplasia are definite to the margin of error inherent in a ory examination, the episodic nature of ease and the usually large inter-subject lity in gland size

t of the 10 patients comprising this eries showed a clear tendency for a low on rate to be associated with a low or ate stimulation level, but a normal on rate with submaximal stimulation fference in the secretion rate of the diseased ie healthy glands of corresponding size ferent stimulus levels is so large that it feasable that there is an organic cause difference in the glands' reaction It is, er, inconceivable that this factor is pri- due to the demonstrable morphological ions in the gland parenchyma, since the 1 of reaction to the different stimuli would resumably be uniform

more likely to be due to a disturbance nervous regulation of the gland function been demonstrated experimentally in the it an increased sympathetic tone inhibits rasympathetically elicited salivation and land cells deprived of their parasympa- control become hypersensitive to certain al substances such as adrenalin and enalin (Richins & Kuntz, 1953, Emmelin, The function test would seem to indicate e hyperplasia is due to increased sensitivity cells resulting from increased sympathetic nce (possibly via a stress factor) This sed influence may at a low level of stimula- artly block the parasympathetic system but omaximum stimulation level be overcome ort for this hypothesis is found in experi- in which sialosis was induced in serous ry glands in the rat (Selye et al, 1961, t, 1962, 1964, Wells & Peronace, 1964), 1966, Ohlin, 1966) Stress can elicit hologically demonstrable reactions in sali- glands as has been shown by, among others, h & Seifter (1948) Seifert and Pohto have ested the following mechanism underlying rplasia in hyperstimulation, isoprenalin certain other biogenic catecholamines disturb the protein synthesis in the serous salivary glands and produce an enlargement of the acinar cells by increasing the endoplasm and vacuole formation in the granules and Golgi apparatus

The salivary glands are to a marked degree species specific and observations on one species are not always applicable to another Even so, the possibility that catecholamines exert an influence on the serous salivary glands in man cannot be excluded, and there is evidence of similarities in the histological and histochemical features of sialosis induced in animals and spontaneous sialosis in man A disorder of the vegetative nervous system—due to, for instance, stress—may therefore be one causal factor in certain forms of sialosis It has been established that the salivary glands in man react to stress and modify the amount of cholinesterase-like substances and electrolytes in the saliva (Prader et al, 1955 Giddon & Lisanti, 1962) In the rat, stress causes a reduction in the number of granules in the salivary gland acini (Roseman et al, 1960)

A careful examination of the patient's social situation may be one approach to the cause of asymptomatic parotid hyperplasia and provide a guide to both diagnosis and treatment

ZUSAMMENFASSING

Bei zehn Patienten mit der Diagnose Parotishyperplasie U N S wurde die sekretorische Kapazitat der Parotis speicheldrusen genau untersucht Verschiedene Grade von Geschmacksreizung wurden verwendet Bei einer moderaten Reizung war die Speichelsekretion bei acht von zehn Patienten reduziert Bei submaximaler Reizung war die Sekretion normal Die Resultate werden diskutiert Ein erhohter Sympatikustonus wahrscheinlich auf Stress beruhend wird als Ursache von Parotishyper plasie U N S angegeben, aber es existieren auch andere Ursachen

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A Wiberg, M D
Dept of Otolaryngology
University Hospital
S 901 87 Umeå 6
Sweden

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RECENT ADVANCES IN MICROSURGERY OF THE MAXILLARY ANTRUM

W V Reynolds and E C Brandow, Jr

From the Department of Otolaryngology, Columbia University, New York, and the Department of Otolaryngology, Union University, Albany, New York, N Y, USA

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t A new and more detailed approach to the ry sinus is described It has the advantage of ng the canine fossa and potential dental trauma rocedure is performed intranasally and is ac- ied by less morbidity than with the Caldwell-Luc on The opening into the sinus is small, thus zing bone removal However, the interior of the can be visualized sufficiently well to justify the ure A sinuscope and operating microscope sate for the diminished exposure In the majority s antral surgery is carried out for chronic sinus and this procedure conforms to basic principles quate drainage and exteriorization of the sinus nasal cavity

gical treatment of chronic maxillary sinu ie Caldwell–Luc and the intranasal antro- have been standard operations for many and are still widely used However, these lures have shown certain limitations and dity

purpose of this paper is to present a new iore appropriate approach to the maxillary employing the operating microscope and iuscope The operating microscope, which volutionized otological surgery, and more ly improved the caliber of endolaryngeal y, has been neglected in surgery of the lary sinus Only in the past few years has n applied to surgery of the antrum

: Caldwell Luc or radical antrum operation s its main advantage a good operating field problem with this procedure is potential ige to the teeth and sometimes considerable idity This operation requires an incision gingivo-labial sulcus almost 2 inches in To expose the anterior wall of the maxilla and canine fossa entails considerable dissection and elevation of the soft tissues of the cheek, with exposure and retraction of the infra-orbital nerve This often results in considerable postoperative swelling of the cheek and, not infrequently, numbness over the distribution of the nerve The more important problem results from removal of bone in the canine fossa, which may result in damage to the teeth Sometimes bone is taken down so close to the teeth that occasionally the apex of a tooth has been inadvertently violated This approach does not interfere in the same degree with the nerve or blood supply of the teeth

A brief review of the innervation of the upper teeth is appropriate (Fig 1) All of the upper teeth are supplied by branches of the maxillary nerve One of the main nerve supplies consists of the infra orbital nerve which comes down through the anterior wall of the maxilla

Harrison reviewed the radical antrum surgery in a series of 96 patients, and found temporary paresthesia in over 70% of cases There were 16 patients who had permanently insensitive teeth The recovery of sensation that occur in most teeth is explained by the fact that all three superior alveolar nerves contribute to the alveolar plexus The blood supply of the upper teeth is often irreparably damaged when radical surgery is employed We have also seen many patients who have complained bitterly post-operatively, and have known dentists who have reported missing apices on extracted teeth in patients who had undergone Caldwell–Luc surgery

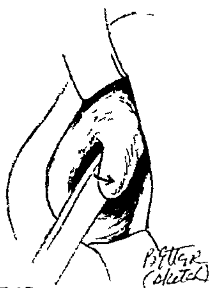


One of the objectives of the Caldwell-Luc operation has been the removal of the membranous lining of the antrum though experience has shown that once adequate drainage has been established, diseased sinus mucus membrane will usually heal, and need not be removed In most cases the establishment of an antrostomy is all that is required

The intranasal antrostomy operation first described by John Hunter and popularized by VanAlyea is difficult to perform well It is a blind procedure which is accompanied by considerable bleeding and does not allow for inspection of the sinus contents The antrostomy window often tends to close rapidly Many prominent authors have recommended that the intranasal operation should be abandoned

As a result of the drawbacks with these procedures a number of other approaches to the maxillary sinus have been suggested Harrison recommends a middle meatal approach with abandonment of the intranasal antrostomy beneath the inferior turbinate, and only occasional use of the Caldwell Luc The authors [illegible] the middle meatal approach to chronic [illegible] sinus disease does not provide depend[illegible] age and will interfere with the natur[illegible] Greenfield has called attention to the [illegible] physiological functions of the sinus Sanderson has described the use of [illegible] plastic flap from the anterior antral [illegible] canine fossa through which he intro[illegible] sinuscope However, this is of no [illegible] advantage since it also violates the [illegible] blood supply to the teeth

Our approach to the maxillary sinus [illegible] nasally by means of a vestibular incision [illegible] through the ascending process of the [illegible] The canine fossa is not violated and [illegible] nent antrostomy remains to provide [illegible] Through the opening a sinuscope can be [illegible] duced for complete visualization and [illegible] and the operating microscope can also be [illegible] to inspect the antrum and aid in the [illegible] the anteronasal wall from before back[illegible]



tions

dications for our new approach are as s

onic sinus disease
gnostic examination
psy of tumours
noval of polyps

5 Removal of irreversibly diseased areas of mucus membrane
6 Removal of tooth roots or other foreign bodies

Procedure

The stages of the operation are as follows

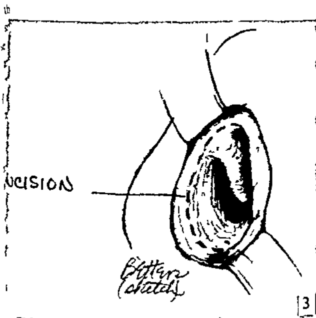
1 The innervation of the anteronasal wall is blocked with Xylocaine 1% and Epinephrine 1–30 000

2 The inferior turbinate is infractured (Fig 2)

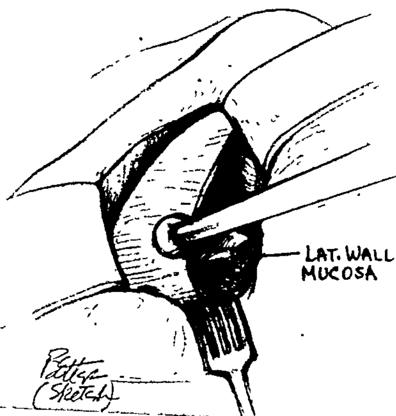
3 A hockey stick incision is made along the anterior edge of the ascending process (Fig 3) of the maxilla from the anterior attachment of the inferior turbinate downwards to the nasal floor

4 A lateral incision is then made to the periosteum from the vertical incision below the attachment of the inferior turbinate about 1 5 cm long, curving downwards to the nasal floor Using a periosteril elevator, a flap is raised (Fig 4)

5 Using a drill, the maxillary antrum is entered anteromedially and the antero-nasal wall is taken down under direct vision, using a Zeiss microscope (Fig 5) The edges of the new opening are rounded off The sinuscope is inserted and the sinus cavity carefully examined,



ELEVATION OF
MUCOSA AND
OF ANTERO-

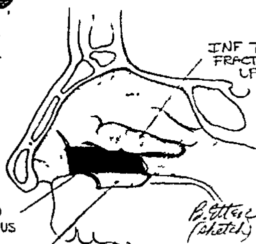




6

7




INF TURBINATE
FRACTURED OUT AN
UPWARD FOR BET
EXPOSURE
INDOW
ITO SINUS
MUCOSAL
FLAP

8



irrigated and suctioned Photography is carried out, and biopsies may be taken (Figs 6 and 7 demonstrating an antral lesion)

6 Using the microscope and dental drill, the anteronasal window is created from before backwards under direct vision (Fig 8)

7 A plastic Teflon prosthesis of variable size may be inserted to maintain the opening and facilitate further irrigation or manipulation without discomfort (Fig 9)

8 The flap is replaced, and the inferior turbinate is moved to the original position

9 If a prosthesis is used it should be removed after about 2 months

RESULTS

We have carried out 70 operations of this kind and although the technique originally seemed difficult, most of the problems have been solved Following the surgery there is some dental numbness but its duration is short lived

CONCLUSION

A new operation is described which is less traumatic than the Caldwell-Luc procedure and more effective than the intranasal antrostomy in surgical treatment of chronic maxillary sinus disease The antrum is approached through an intranasal vestibular incision entering the lower part of the ascending process of *the maxilla* Through this opening the sinuscope is [illegible] and the microscope is also used to [illegible] adequate anteronasal window

Finally, I deem it appropriate to [illegible] quotation from a well known orthope[illegible] book *Extensile Exposure* by A K Henry [illegible] was one of my professors at medical school for whom I have great respect

> "'tis not so deep as a well nor so [illegible]
> a church door but [illegible] 'twill serve

ZUSAMMENFASSUNG

Es wird eine neue Operation fur chronische hohlenerkrankungen beschrieben die wen[illegible] ist als die Caldwell Luc Operation und erfolg[illegible] die intranasale Antrostomie Wenn die Zug[illegible] der Gewebe unzureichend ist ist eine radikale[illegible] tion angebracht (L L F)

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W V Reynolds M D
Dept of Otolaryngology
The Mary Imogene Bassett Hospital
Cooperstown N Y USA

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DAMAGE TO THE STRIA VASCULARIS IN THE GUINEA PIG BY ACUTE ATOXYL INTOXICATION

M Anniko and J Wersall

From the Department of Otolaryngology Huddinge Sjukhus and King Gustav V Research Institute Stockholm Sweden

(Received April 1 1975)

t The aim of the present study was to show the ogical degeneration at the ultrastructural level maging the stria vascularis experimentally The sions after acute atoxyl intoxication occur after ' hours and begin as a degeneration of both the l and the intermediate cells whereas the basal nain mainly unaffected The severely damaged or intermediate cells may become loosened from ³i vascularis and rejected from it into the en atic space Under such conditions the basal cells the surface facing the endolymph although this ery rarely Initially there may be a slight bulging ⁷sner s membrane but soon the membrane depressed and sometimes a total collapse oc lh Reissner s membrane flattened over the tec mbrane against the organ of Corti It is only hat Reissner s membrane touches the strial sur ochondrial degeneration with formation of intra idrial inclusion bodies is an interesting early i the damage pattern

l Alphonse Corti described the stria aris as a capillary network closely nded by epithelial cells Nowadays it l known that three different types of ial cells are found in the stria the dark iophil or marginal) the light (chromo or intermediate) cells and the basal v Fieandt & Saxen 1937 Engstrom 1955 Smith 1957 Bairati & Iurato On the basis of the ultrastructural fea t may be assumed that the stria an ex y highly differentiated tissue is able to perform a selective secretory activity and to a lesser extent, some resorptive function (Spoendlin 1967)

In 1938 Hallpike & Cairns were the first to describe the histological picture in Meniere s disease They found gross dilatation of the scala media and degneneration of both Corti s organ and the stria vascularis

Recently the functional pathology of the stria vascularis has aroused renewed attention following the demonstration of strial damage in experimental animals treated with etha crynic acid by Quick & Duvall (1970) Na kai (1971) also reported strial changes due to ethacrynic acid

The toxic effect of the arsenic compound atoxyl on inner ear structures was described by Miayamoto (1931) He found histopatho-logic abnormalities in the organ of Corti and the stria vascularis Nakamura (1935) Ozeni (1937) and Ruedi (1951) confirmed these findings The effects of arsacetin (acetylated atoxyl) were first studied by Causse et al (1940) who recorded loss of the Preyer s reflex and the cochlear microphonics and de layed vestibular disturbance Similar findings were obtained by Leonard et al (1971)

Johansen (1953) reported marked histopatho-logic changes after arsacetin administration involving the organ of Corti and the stria vas cularis

The purpose of the present study was to

ırk was supported by grants from the Karol nska t and from the Swed sh Med cal Research Council o 1²X 7²0)

Table I *Diagram illustrating the doses injected into the guinea pigs of the experiment* [illegible]

No	Mg/kg at each injection	Number of injections	Injected during (days)	Totally mg/kg	Killed after the last injection (days)	Preyer reflex
20	70	3	2	210	1	–
52	70	2	¼	140	¼	–
53	100	1		100	1	(+)
54	70	2	¼	140	¼	(+)
55	140	1		140	1	–
63	140	1		140	¼	–

investigate the toxic effects of atoxyl on the stria vascularis with special regards to the question whether atoxyl is a suitable substance for experimental studies of strial function and pathology

MATERIALS AND METHODS

Nine young, healthy guinea pigs, with normal Preyer's reflex, weighing aroung 250–350 grams, were used for the experiment Six animals were injected with atoxyl The control group consisted of 3 healthy, non injected guinea pigs

Administration of atoxyl

Each animal was given a 2% solution of atoxyl in sterile water subcutaneously during a certain period of time The dosage varied between 70 and 140 mg atoxyl per kg body weight The total dose ranged from 100 to 210 mg per kg body weight The duration of administration varied between 6 hours and 2 days and the survival time before decapitation between 6 hours and 1 day after the last injection (Table I)

Morphological procedures

For microscopy, specimens were taken according to the technique of Wersall (1956) with decapitation of the animal The te[illegible] bones were removed, and an openi[illegible] made in the bulla tympanica The stap[illegible] extracted, the round window me[illegible] opened, and a small opening made at th[illegible] to allow sufficient penetration for fi[illegible] The cochlea was perfused with 2% b[illegible] osmium tetroxide solution through the [illegible] in the apical coil of the cochlea The sp[illegible] was immersed for 2 hours in the fixativ[illegible] then dehydrated in alcohol, and emb[illegible] epon 1 μm thick sections stained [illegible] luidene blue, were studied in th[illegible] microscope Thin sections were ex[illegible] the electron microscope after stain[illegible] uranyl acetate and lead acetate

RESULTS

Structural alterations may be obser[illegible] some parts of the stria vascularis even [illegible] mately 12 hours after the administrati[illegible] atoxyl After 24 hours severe degene[illegible] occurs in all the turns of the cochlea (F[illegible]

After treatment with 70 mg atoxyl p[illegible] body weight, and repeating the same d[illegible] ter 6 hours the animal was killed 12 hour[illegible] the first injection The apical turns w[illegible] verely affected whereas the basal coil sh[illegible] only minimal changes The same change[illegible]



(a–d) Different stages of strial degeneration. Light microscopy. Magnification 280×. The stria vascularis initially become swollen (a: 3rd coil, animal No. 20) and marginal and intermediate cells are rejected from the strial surface (b, c: 3rd coil, animal No. 5?). The position of Reissner's membrane is altered and it becomes depressed and lies over the organ of Corti in the final stage (d: 3rd coil, animal No. 54).



Fig 2 Heavily degenerating intermediate (*I*) cells in the stria vascularis The blood vessel (*B*) is blocked by red blood cells (3rd coil, animal No 20) EMG

ved when 140 mg atoxyl per kg body t were administered, and the investiga- was made 24 hours after the injection rvations after only 12 hours showed an rt normal morphological structure An in- h of 100 mg atoxyl per kg body weight, he specimen studied after 24 hours, d only minimal degeneration in the stria laris, except in the apical coil Severe eration of the stria vascularis occurred 70 mg atoxyl per kg body weight was istered three times within 36 hours, and uinea pig killed 48 hours after the first on In these specimens the stria of all the was so severely damaged that no coil was ved to predominate The damage areas e localized or diffused Lesions occurred oth the blood vessels in the stria vas-, and the spiral prominence, the latter e most frequent site Among the three of strial cells, the marginal and in- diate cells were most frequently affected)

degeneration of marginal and in- diate cells followed the same pattern nt degrees of vacuolization appears Figs 2 and 3 b) which may become so ive that almost the whole cell is filled up uoles, and the degenerating cell organel- l nucleus are pressed towards the periph- arts of the cell body The normal cell ance, with cytoplasm densely packed itochondria, especially in the basal part, Golgi membranes, was lost at an early during degeneration The normally ir- lr, chromatin rich nucleus was the last onent to disintegrate by swelling, chro- fragmentation and, finally, by rupture of uclear membrane The mitochondria go swelling, and accumulation of dense ial occurred between the cristae (Fig One or more such accumulations can be in almost every damaged mitochon- , but they occupy only a minor portion These changes occur relatively soon in itial phase of mitochondrial degeneration the cristae mitochondriales are still often preserved, although they are irregular and fragmented In some cases the dense material occupied a major part of the mitochondrial volume In a few cases mito- chondrial degeneration, with formation of lamellated bodies, occurred

While these degenerative processes were taking place there was a tendency for the re- jection of the severely damaged cells from the stria vascularis into the endolymph (Fig 4 a, b) Not all the marginal cells, however, that become damaged undergo all the phases of degeneration before they are loosened from the stria A cell may be rejected also at an earlier stage, whereas some cell organelles and fragments of the nucleus may still be rec- ognized

The lining facing the endolymph will, there- fore, consist of both marginal and intermediate cells and, to a lesser degree, also of basal cells The loosening of the marginal cells from the stria vascularis is more manifest towards the spiral prominence, but is also observed around the vessels in the stria to a great extent The area around the insertion of Reissner's membrane is not so severely affected Some of the rejected cells are so severely damaged that it is impossible to recognize their origin (Fig 5) There are findings which indicate that the intermediate cells may be affected first In several serial sections starting at the stria vascularis and proceeding towards the spiral ganglion rather severely degenerating in- termediate cells may be observed, whereas the processes of the marginal cells appear to be only slightly damaged

In the cell cytoplasm of many intermediate cells there are accumulations of colloid like, dense material (Fig 3 b) of the size of several mitochondria Nothing of the ordinary endo- plasmatic reticulum or ribosomes can be observed Severely damaged intermediate cells are rejected from the stria vascularis in the same way as in the marginal cells The melanin pigment participates in the rejection process either as a component of the rejected cell or it may also be found in the endolymph



Fig 3 (a) Intermediate cell with vesiculation and degenerating mitochondria (*M*) containing electron-dense material (3rd coil animal No 20) EMG (b) Intermediate (*I*) cell with lipoid like material (*L*) in the cytopl siculation and mitochondrial degenerat on (3rd coi No 20) EMG

(a) Advanced stage of the rejection of heavily rating marginal and intermediate cells (2nd coil No 57) EMG (b) Early stage of the rejection of a moderately damaged marginal (*M*) cell The blood vessels (*B*) are blocked by red blood cells The basal cells (*BC*) appear normal (2nd coil animal No 54) EMG



Fig 5 Loosening of heavily degenerating marginal and intermediate cells from the surface of the stria vascularis The basal cells BC remain unaffected (3rd coil No 57) EMG

.., probably as the result of a completely tegrated cell

.e flat, tightly packed basal cells, with sometimes gigantic intracellular vacu- are very little affected by acute atoxyl ication However, not until the marginal the intermediate cells have degenerated become loosened from the stria vas- is, and the adjacent marginal and in- ediate cells are unable to stretch and fill ie missing cell spaces, do the basal cells me involved in the lining towards the en- nphatic space

e strial vessels are often blocked by e accumulations of blood cells In a few l capillaries, small lamellated bodies may served near the nucleus in the endothelial In acute atoxyl intoxication an intercellu- iace around the blood vessels in the stria ilaris is a rather constant finding (Fig 6) ich space is found in the control group ring the early stages of atoxyl administra- Reissner's membrane initially becomes ly bulging, but this stage is soon followed pression of the membrane, so that it be s flattened over the tectorial membrane st the organ of Corti When Reissner s orane is collapsed it covers the tectorial orane and the Hensen cells, but it very m touches the stria vascularis itself (Fig lthough all the coils of the cochlea are ed, the findings are more pronounced at ical coils Between this extreme position issner's membrane, as described above, its normal *position all kinds of in* ediate stages may be found

DISCUSSION

present investigation shows that the stria ularis in atoxyl treated guinea pigs under- different stages of degeneration of the ginal and the intermediate cells The stria initially appear normal under the light oscope but under the electron micro- e morphological alternations are discernible Among the strial cells the earliest and most frequently affected are both the marginal and the intermediate cells and it has so far not been possible to assert with certainty which cell type degenerates first However, in some specimens the intermediate cells are more severely affected than the marginal cells

Both marginal and intermediate cells may become loosened from the stria vascularis and float out into the endolymph At the same time, pigment is also liberated, so that it flows out into the endolymph Here and there, cells may be seen to burst and discharge their content of pigment The degeneration of the stria vascularis may occur in all parts of the true stria vascularis, although there is a predilection for the zone near the spiral prominence, and that near the blood vessels The reason for the vulnerability of these areas is not clear but it is likely that there may be a high concentra tion of the substance around the vessels

Mygind & Dederling (1932) and Hallpike & Cairns (1938) were the first to describe the distention of the cochlear duct in histological specimens, as evidenced by the bulging of displaced Reissner s membrane These findings have been confirmed by Hallpike & Wright (1940) Rollin (1940), Lindsay (1942) and Alt man & Fowler (1943), so that the term 'en dolymphatic hydrops' is generally accepted as a synonym for Meniere's disease In the acute atoxyl intoxicated guinea pig there is a tendency for a slight hydrops to occur initially but the slightly bulging Reissner s membrane *is very* soon depressed, and subsequently it collapses to a considerable extent Altman (1961) stated that the dilatation of the endolymphatic system appears to be the result of an increased endolymphatic pressure probably due to a change in the chemical constitu tion of the endolymph by an accumulation of molecules causing a light osmotic pressure in the endolymph In acute atoxyl intoxication the initial phase with hydrops is extremely short in relation to the phase of strial cell degeneration

Quick & Duvall (1970) reported changes in

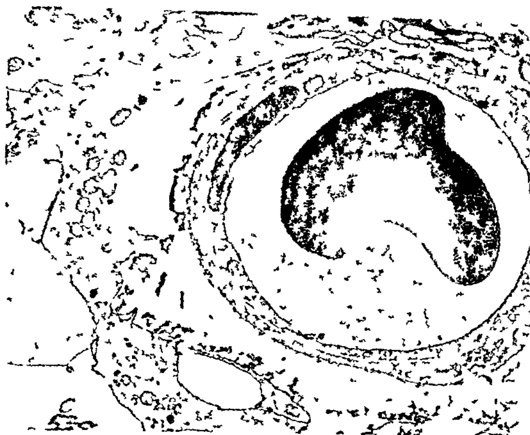


Fig 6 Strial blood vessel with surrounding intercellular space (I) (2nd coil animal No 70) EMG

the stria vascularis after high doses of ethacrynic acid whereas smaller doses did not produce any anomalies There was an increase in the thickness of the stria vascularis due to intracellular and extracellular edema The marginal and the basal cells looked normal The intermediate cells were either completely destroyed or in an advanced stage of atrophy The melanin granules normally contained within the intermediate cells seemed resistant to atrophy

Nakai (1971) reported however that ethacrynic acid intoxication mainly affected the marginal cells of the stria vascularis Contrary to the damage to the sensory cells in the organ of Corti the changes in the stria vascularis had disappeared in cases that were observed for long periods after final administration Whereas the ethacrynic acid degeneration of the strial cells apparently is reversible in the early stage of toxic reaction t[illegible] found after atoxyl intoxication are irreversible type

Kimura & Schuknecht (1970) fo[illegible] tients with Meniere s disease that th[illegible] and most frequent changes were in t[illegible] nal cells by decrease in cytoplasmic reduction in the number of mito[illegible] pinocytotic vesicles and rough endop[illegible] reticula The intermediate cells were [illegible] quently effected but similar changes o[illegible] As tha marginal cell layer thinned intermediate cells grew and occupied [illegible] jor portion of the stria The basal ce[illegible] least affected When the marginal [illegible] termediate cells disappeared the bas[illegible] often remained intact thus the endoly[illegible] surface emerged The general find[illegible] as the thickness of the stria vascula[illegible] creases the extent of the pathology inc[illegible]

ıar findings, concerning the basal cells, made in connection with atoxyl treat

ding (Kichuchi & Hilding, 1965, Hilding 1967) reported that the mitochondria, omes, Golgi complexes and endoplasmic lla are seen, within normal range, in the vascularis *up to 4 weeks of age in the* r 1 mouse However, these structures started to become less numerous, and erative changes appeared Leonard et al) studied the effects of damage to the of Corti and the stria vascularis, on en- phatic cationic changes due to treatment arsacetin Arsacetin was chosen in view established ability to damage both the vascularis and the organ of Corti by alter- he components of the scala media Depres- of cochlear microphonics (CM) was in many animals The histopathological rance of the organ of Corti was abnormal instances in which the Preyer s reflex or ere altered In animals with depression endolymphatic, direct-current potential light microscopic investigation showed ie stria vascularis appeared to have been ed In the total histopathological study, olated instances of damage to the stria ularis alone could be observed Tasaki & poulous (1959) assumed that cochlear phonics (CM) originate from the hair of the organ of Corti, and that the en phatic, direct-current potential (EP) ates from the stria vascularis Investiga by Matschinsky & Thalmann (1967) and y by Kuijpers (1969) have clearly shown he marginal cells are involved in the re l of sodium from the endolymph and are emitting potassium into this fluid there estoring and maintaining the important rolyte composition of the endolymph sturbance of the metabolism of the margi ells which are assumed to be engaged in transport (Spoendlin, 1967) must in- re with endolymph production and to a r degree, also with reabsorption

ie subsequent degeneration of the margi- nal cells, resulting in their rejection, will thus block the endolymph circulation, and the electrolyte balance will collapse This must interfere with the position of Reissner's membrane, which becomes depressed A sim- ple rupture at some point cannot result in the general depression of the whole membrane in *all its coils It is apparent that a passage of* fluid through Reissner's membrane is not suf ficient to allow for a normal location of this membrane Lawrence (1964) established that perforation of Reissner's membrane at single points causes no damage to the stria vas cularis, but the perilymph is absorbed at the local level around the perforation However, damage was noted in the cells, on the basilar membrane, nearest to the perforation

As expected, primary lesions of the stria, caused by a substance reaching the stria, might be supposed to appear via the blood stream equally distributed in all turns of the cochlea However, in several guinea pigs the severest changes were found in the apical coils, whereas the basal turn, although dam aged was not so severely affected Similar findings were described by Kimura & Perlman (1958) and by Bernstein & Silverstein (1966) by the obstruction of labyrinthine vessels Ki mura & Perlman (1958) found that after arterial obstruction the stria vascularis appeared to become partly detached from the spiral liga ment as one unit without edema, hemorrhage or dilation of the vessels After venous ob- struction the stria did not become partly de tached but edema vascular dilation, and fragmentation occurred The stria vascularis finally disappeared and was replaced by low flat epithelium The stria in the region adjacent to the round window changed more slowly The position of Reissner's membrane re mained unchanged throughout However, Bernstein & Silverstein (1966) found a collapse of Reissner s membrane, especially in the api cal turns

Strial atrophy of the ageing ear is well documented by Schuknecht (1955) and by Schuknecht & Igarashi (1964) It is char-

acterized by degenerative changes, however, which begin at the basal end of the cochlear duct and proceed towards the apex, affecting almost equally and simultaneously the various structures within the duct

The finding of an intercellular space sometimes around the blood vessels in the stria vascularis, in connection with atoxyl treatment, is rather obscure Spoendlin (1967) summarized the general opinion concerning the stria vascularis, and stated that such findings are usually regarded as due to fixation artefacts There was no such space, however, surrounding the blood vessels in the control material, although it was treated in the same way in the fixation fluid The term intercellular space is, to some extent, misleading, because arsenicals in general cause damage to blood vessels (Osol & Pratt, 1973) Consequently, the blood vessel is surrounded by blood colloids and electrolytes, hereby causing separation of the marginal-cell extentions from the capillary wall The intercellular space would, therefore, be the result of increased capillary permeability

The changes found in the stria vascularis indicate that the stria vascularis is one primary site of action of atoxyl A degeneration of the stria independent of reason for the degeneration is invariably followed by hair cell damage The objective of further studies on the ... will be to correlate strial damage with ...ar hair cell degeneration in various parts of the cochlea and its physiological significance

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The authors wish to express their appreciation to Mrs Marie Louise Spångberg for skilful technical assistance

ZUSAMMENFASSUNG

Die Absicht der vorliegenden Untersuchung ist es auf ultrastrukturellem Niveau die morphologische Degeneration der Stria vascularis nach experimenteller Schadigung darzustellen Die akute Schadigung nach akuter Atoxylintoxikation tritt nach ca 12 Stunden auf und beginnt als Degeneration *der marginalen und intermediaren Zellen* wahrend die basalen Zellen im wesen... bleiben Die stark geschadigten marginalen ... diaren Zellen konnen von der Stria vascularis in den endolymphatischen Raum abgestosse... solchen Fallen bekleiden die basalen ... Oberflache zur Endolymphe wenngleich d... selten vorkommt Nach anfänglicher leichter ... der Reissnerschen Membran kommt es ... Senkung und in manchen Fallen zu einem vol... *der Membran* wobei die Reissnersche Mem... Membrana tectoria gegen das Cortische ... geflacht ist Nur in seltenen Fallen beruh... nersche Membran die striale Oberflache E... tion der Mitochondrien mit Bildung vo... chondrialen Einschlußkorperchen ist ein ... Fruhbefund des Schädigungsmusters

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Matti Anniko M D
Dept of Otolaryngology
Huddinge Sjukhus
S 141 86 Huddinge
Sweden

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OPTOKINETIC NYSTAGMUS AFTER CEREBELLAR UVULONODULECTOMY IN SQUIRREL MONKEYS

M Igarashi, H Miyata,[1] Y Kato,[1] W K Wright and J K Levy[2]

From the Department of Otorhinolaryngology and Communicative Sciences Baylor College of Medicine Houston Tex USA

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Abstract After cerebellar uvulonodulectomy in squirrel monkeys (*Saimiri sciureus*) a change occurred in the optokinetic nystagmus in that there was a slow phase eye speed decline at the stimulus speeds above 90°/sec The difference was statistically significant when pre and post ablative comparisons were made This result supports the usefulness of the optokinetic nystagmus examination to diagnose conditions which involve posterior inferior vermis of vestibulo cerebellum The optokinetic after nystagmus depicted a similar decline postoperatively

As mentioned in Adler's textbook (1959), the railroad (optokinetic) nystagmus was studied and used clinically by Barany in 1920 (published in 1921–22) He observed the optokinetic nystagmus (clear pursuit move ... when a black striped roll was rotated in ... of an infant s eyes, even a few hours after his birth Also, he saw a few cases of damage to the frontal area and had an impression that the optokinetic nystagmus was limited in its quick phase

Presently the optokinetic nystagmus examination is widely used in clinics and this test is considered to be useful especially for the diagnosis of central nervous system disease based upon both clinical and ... observations

Cogan (1956) previously pointe... experimental results of the ... nystagmus in animals (such as rabb... applied to humans only with reser... cause of the many differences whi... tween the ocular apparatus of ma... lower vertebrate experimental ... Therefore, it is advantageous to ... human primates whose ocular syst... bles man's for this kind of experim... ermore, Komatsuzaki et al (1969... that the optokinetic nystagmus of ... monkey appeared to be similar to th... although optokinetic after nystagmu... prominent in the monkey

The purpose of the present study ... vestigate the change in optokinetic n... following partial ablation of ... cerebellum (cerebellar uvulonodulec... the squirrel monkeys The informa... tained will relate to the clinical dia... diseases involving this particular regio...

This study was supported in part by the National Institute of Neurological Diseases and Stroke (grant NS-07237 P01 NS 10940 K03 NS 38619) and the McFadden Charitable Trust Fund

SUBJECTS

Eleven squirrel monkeys (*Saimiri sc...* were used for the present study The... healthy young adults about 2 years ...

randomly selected from several different ıal groups (with no preference as to their The subjects were well aclimated to our atory conditions prior to the initiation of ımental procedures

PROCEDURE

squirrel monkey subject was restrained ut anesthesia Application of pressure to eck region was avoided Then, the mon was placed at the center of a 60 cm ter illuminated white cylinder The cyl- which can be rotated by a motor has 16 al black stripes that are 1 5 cm wide and ly separated Subdermal platinum needle odes were implanted at the outer canthi der to record the horizontal eye move- The optokinetic stimulus speed used -200°/sec with 1°/sec² constant angular eration When the stimulus speed ed 200°/sec, the illumination was in- aneously eliminated in order to record the inetic after nystagmus

ernal stimuli (a remote operated tail ing and a loud noise applied from a horn) given to the subject randomly every 2–3 ds during the entire optokinetic gmus and optokinetic after nystagmus ding Amphetamine which is a reticular ation stimulant, was not used in this of experiments Both clockwise and er clockwise stimulations were used

operative optokinetic nystagmus ine measurements were repeatedly per ed with an interval of about 7 days (Pre- Upon acquisition of baseline data, the keys were anesthetized with sodium obarbital and the area of the cerebellar a and nodulus (Larsell's IX and X) were roached through the posterior cranial fos Uvulonodulectomy was performed by gen spiration in 7 monkeys, while the other 4 keys were used for sham control opera- In the control group the posterior in r cerebellar vermis was exposed but no ablation was performed

The post-operative optokinetic nystagmus was recorded on the fifth post operative day (Post Op-I) Later, the examination was repeated after the locomotor equilibrium function (measured by the squirrel monkey rail test) regained the pre-operative level (Post Op-II), the average of 88 post operative days (the latest test date was 114 days post-operation)

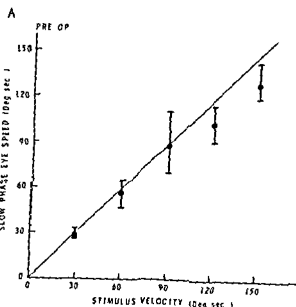
The slow and quick phase eye speeds of optokinetic nystagmus at the stimulus speed of 30°/sec, 60°/sec, 90°/sec, 120°/sec, and 150°/sec were analysed All basic numerical values were obtained by averaging three typical nystagmic beats at each level of these stimulus speeds The numerical values obtained by both clockwise and counter-clockwise stimulations (which showed no distinctive asymmetry) were averaged The slow and quick phase eye speeds of optokinetic after-nystagmus were obtained by averaging five typical nystagmic beats immediately following the cessation of the optokinetic stimulus

At the end of the experiment, all subjects were sacrificed in order to morphologically confirm the extent of lesions

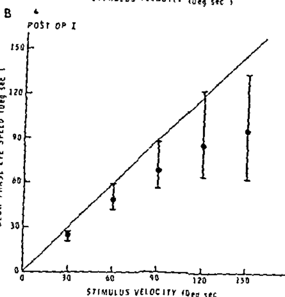
RESULTS

The slow phase eye-speed of the pre operative status (Pre-Op) (average of repeated measures) early post operative status (Post Op I), and the late post-operative status (Post-Op II), were displayed in Fig 1 A–C The results were statistically studied at the 30°/sec, 60°/sec, 90°/sec 120°/sec, and 150°/sec levels of optokinetic stimulus speed, by Duncan's multiple range test Both Pre Op vs Post-Op I comparison and Pre-Op-II comparisons exhibited statistically significant differences at 90°/sec 120°/sec, and 150°/sec stimulus speed points, while the comparisons at 30°/sec, and 60°/sec did not show any significant differences (Table I) A comparison of average graphical displays (Fig 1 A–C) however exhibited a slight lowering of adaptation limit

A



B



C

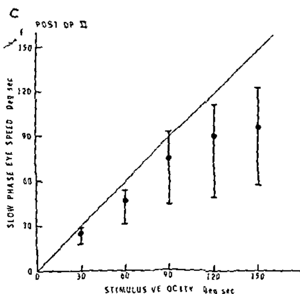


Table 1 *Optokinetic nystagmus Slow eye speed (post uvulonodulectomy)*

Statistics by Duncan s Multiple Range Test
S Significant difference NS Non-significant
$n=7$

	Pre-op	Post-op I	Po
30°	I←——NS——→I	←——NS——	
	I←———————NS———		
60°	I←——NS——→I	←——NS——	
	I←———————NS———		
90°	I←——S——→I	←——NS——	
	I←———————S———		
120°	I←——S——→I	←——NS——	
	I←———————S———		
150°	I←——S——→I	←——NS——	
	I←———————S———		

(point of non linearity) after uvulon tomy Post operative increase of inter ual variance was also noticed only at s speeds above 90°/sec Even though recovery tendency was noticed whe 1 B and 1 C are compared statistic parison between Post Op I and Po showed no significant difference (Ta

The overall comparisons of quick ph speed between three stages, perfor identical analysis failed to depict an cant difference statistically, even shor uvulonodulectomy Dysrhythmia or n inversion were not found Irregul which was actually a mixture of sacc movement was seen in only one out of cases Amplitude was not consiste post operatively a slight tendency of tude increase was noticed Frequency generally decreased

Fig 1 (A) This figure displays the slow phase e at 30°/sec 60°/sec 90°/sec 120°/sec and 150°/sec velocity points before the cerebellar uvulonod $n=7$ The vertical axis indicates the slow phase ey and the abscissa represents the optokinetic speed Vertical bars indicate the range of obtained ical values (B) The slow phase eye speeds short the operation (C) The slow phase eye speeds after the operation Locomotor dysequilibrium was ered by this time

r sham control subjects demonstrated cline at all in any of the optokinetic gmus parameters even shortly after nodulectomy On the contrary, the aver- sult from these four monkeys demon d a slight response increase

examining optokinetic after nystagmus, ow phase eye speed depicted a statisti significant difference only between Pre- d Post Op I comparison The compari quick phase eye speed failed to show ignificant difference among different arisons

surgical lesions in all seven experimen- mals used for the analysis were properly (not involving neighboring areas), even the extent of lesions was slightly vari Three other subjects with inadequate le were not included in the present study

DISCUSSION

sefulness of the optokinetic nystagmus nation as a diagnostic tool for central is system disease was emphasized by & Kornhuber (1964) Ino (1970) reported he pathological findings of horizontal netic nystagmus are frequently ob- in cases with central nervous system es, especially those involving the brain According to his data, more than 50% of ses with central nervous system lesions d some abnormality of optokinetic gmus The present experimental result ms the post uvulonodulectomy decline okinetic nystagmus reported in the pre ry study (Igarashi et al , 1973) was accu The fastigial nuclei project to the regions pontine reticular formation near where ontal eye movement are believed to be ated and stimulation of these nuclei in l horizontal eye movement (Cohen & stein, 1972) In different animal species it een shown that stimulation of the fastigial or of the vermis which overlies the al nuclei mainly induces horizontal eye ent The results of the present experi ment ablating uvula and nodulus (vestibulo-cerebellum) which overlie the fastigial nuclei, indicated change in the slow phase eye speed of the horizontal optokinetic nystagmus in squirrel monkeys

The classical Dow's study in the monkey (Dow & Moruzzi 1958) concluded that the nodulus as well as the uvula sent fibers to all four main vestibular nuclei on the homolateral side According to Brodal (1969), a subsequent study of Angaut & Brodal in 1967 confirmed this concept, also it seems likely that the influ ence on nystagmus are mediated by the effer- ent fibers from the nodulus and uvula to the vestibular nuclei Thus the ablation of the muscle proprioceptor-cerebellum oculomotor vestibular nuclei complex and this connection must have an influence on the optic-oculomotor reflex pathway through the neural pathway between the vestibular nuclei and the oculomotor nucleus (Fukuda et al , 1972) Eye muscle proprioceptor cerebellum-oculomotor nucleus pathway must also be involved by uvulonodulectomy

According to Komatsuzaki et al (1969), the slow phase velocity of the optokinetic nys- tagmus was most closely related to the op- tokinetic stimulus speed in rhesus monkeys Based upon this information in the present experiment the slow phase eye speed of hori zontal optokinetic nystagmus was analysed in squirrel monkeys Cohen et al (1973) found that the slow phase eye speed of optokinetic nystagmus returned to the original values 10 months after the labyrinthectomy There may be a possibility that similar recovery may have occurred if the animals in the present study had been permitted to survive longer

Uvulonodulectomy resulted in optokinetic hypo-nystagmus at high stimulus speeds, therefore, the present experimental results may be extrapolated to the acute conditions such as whip lash automobile injury, sudden vascular occlusion which involves the region, and certain forms of war injury, etc On the other hand chronic diseases such as slowly growing tumor or infection, etc may not

necessarily depict this kind of alteration in optokinetic nystagmus According to Tokita's report, however, 5 cerebellar tumor cases (meduloblastoma 2, glioblastoma 1, hemangioblastoma 1, other 1) exhibited low slow phase eye speed and irregular (step like) wave form (Tokita, 1973)

The presence of post-uvulonodulectomy spontaneous nystagmus was very inconsistent Generally the spontaneous nystagmus had a more vertical component than horizontal one Furthermore, even though the presence of spontaneous nystagmus enhances optokinetic nystagmus (when it has a horizontal component), the post uvulonodulectomy optokinetic nystagmus showed a statistically significant decline when compared with that of the pre operative condition

When the pre-operative measurements and the post-operative measurements were compared in control subjects, a slight response increase of the slow-phase eye speed of optokinetic nystagmus was found, even though the testing was performed only infrequently Therefore, the post-uvulonodulectomy optokinetic nystagmus 'decline' represented true ablation effects and not artefactual results

Significant post uvulonodulectomy optokinetic hypo nystagmus was only found when the stimulus speed was higher than 90°/sec, thus this result confirms the importance of the high speed optokinetic stimulus The post-uvulonodulectomy decline of optokinetic nystagmus in this experiment confirms the importance of uvulonodular input to the optically evoked oculomotor response, whichever neural channels are involved The similar reduction of optokinetic afternystagmus following uvulonodulectomy probably indicates that the optokinetic afternystagmus is a reflectory response of the optokinetic nystagmus

ZUSAMMENFASSUNG

Nach Entfernung der zerebellaren Uvulonodulektomie beim Totenkopfaffen (Saimiri Sciureus) erfolgte eine Veranderung im optokinetischen Nystagmus [illegible] eine Verringerung der langsamen Augengeschw[illegible] bei einer Anreizgeschwindigkeit von uber 90° Unterschied war statistisch bedeutend wenn nach der Operation Vergleiche angestellt wurde Resultat bestatigt wie nutzlich die Untersuchung optokinetischen Nystagmus ist um gewisse L[illegible] bestimmen die das hintere unten gelegene Zapfchen im Vorhof des Kleinhirns betr[illegible] Optokinetische Nachnystagmus zeigte eine Abnahme nach der Operation

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M Igarashi M D
Dept of Otolaryngology
Baylor College of Medicine
Houston Tex 77025 USA

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PLANAR RELATIONSHIPS OF THE SEMICIRCULAR CANALS IN MAN

R H I Blanks,[1] I S Curthoys[2] and C H Markham

From the Department of Neurology University of California School of Medicine Los Angeles Calif 90024 USA

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Principal-component analyses were determined nes of points measured from the dissected bony th of ten human skulls resulting in planar equa or each of the six semicircular canals Following igles were calculated between the ipsilateral canal between opposite synergistically acting canal and between each canal and the Reid stereotaxic Results indicated that pairs of ipsilateral canals early perpendicular with the exception of the angle between the anterior and horizontal canal (111°) Pairs of contralateral synergistic canal ormed angles of 19° between right and left horizon l planes and 23–24° between vertical canal pairs horizontal canals formed an angle of 25° with the Reid ntal plane Mathematical equations of the semi canals were used to predict the optimal head for rotational and caloric stimulation

xtensive number of studies have used n subjects to study the stimulus-response cteristics of the semicircular canals In of these, angular acceleration was pro- l by rotation about the major head axes (Melvill Jones et al , 1964, Clark & Stewart, 1968, Oosterveld, 1970, Guedry et al , 1971, also see review by Clark, 1967) producing a combined stimulation of several pairs of canals In other studies, however, subjects were positioned for the purpose of stimulating a single pair of vertical canals (Van Der Vis, 1958, Decker, 1969) Positioning was based on the assumption that the vertical canals were oriented perpendicular to the horizontal canal and formed 45° angles with the sagittal plane For the horizontal canals, and using angular acceleration in the earth horizontal plane, descriptions of head position have ranged from a 30° (Van Egmond et al , 1949, Fitzgerald & Hallpike, 1942) to a 20–25° bent forward position (Graybiel et al , 1948) Other investigators have not described their method of positioning the head but have stated that the horizontal canals were aligned in the plane of rotation (Brown & Crampton, 1964)

Canal stimulation experiments using subjects in a variety of head positions raise the questions of which canals are activated by the stimulus and to what degree It has been known since the time of Ewald that a single canal will be maximally stimulated when rotated in its plane and minimally affected when rotated in a plane perpendicular to the canal

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[1] Planck Institut fur Hirnforschung 6 Frank t—Niederrad West Germany

[2] artment of Psychology University of Sydney y N S W Australia

plane It can be concluded that to stimulate individual canals or pairs of canals properly and to control the amount of stimulation to other canal pairs, the position of the canal planes must be known

A complete description of the human semicircular canals in relationship to the stereotaxic reference system and the internal angular relationships between canal planes has not appeared in the literature Previous studies have been mainly limited to an angular description of a single canal Spiegel & Sommer *(1947) describe the horizontal canal as being* elevated 15–30° above the horizontal plane while Girard (1923) gives a value of 25°±7° for the same angle Other workers, however, have reported smaller angles formed by the horizontal canal with the Frankfurt horizontal plane For example, Kudo (1965) reported 15°87' (sic) and Nishimura (1930) 12 2°

A few authors have described the angular relationships of the vertical canals Colosi & Giannardi (1968) examined the angles formed by the vertical canals and the vertical stereotaxic planes in X ray material These angles were also described by Spiegel & Sommer (1947) and by Mincker (1968)

It is clear from this brief review of the literature that more information is required to define more precisely the position of the semicircular canal planes with respect to skull , This report is concerned with a mathematical description of the human semicircular canal planes with respect to each other and to the stereotaxic coordinate system

PROCEDURES

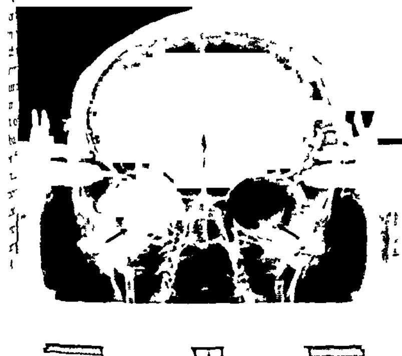
A description of the position of the semicircular canals requires a convenient external frame of reference For this reason the Reid stereotaxic coordinate system was employed This system defines the horizontal plane as one passing through the inferior margin of the orbits (orbitale) and the center points of the two external auditory canals The Reid stereotaxic frontal and sagittal pl perpendicular to the horizontal p' through the center of the external canal and mid sagittal suture Mathematical conventions Hixson et al (1966) to define the system were used throughout this s comparison, measurements of the horizontal plane (Ohr Augen taken This reference system differs Reid in that the horizontal plane sequently the vertical planes, are de the above orbital points and the margin of the external auditory canal

Material consisted of ten racially man skulls (Carolina Biological Burlington, N C) which were free pathology or bone deformities Sk secured to a Talairach human stereo strument (Schaltenbrand & Bailey two ear bars placed in the center of t nal auditory canal and two eye ba made contact with the inferior orbits These four craniometric poin the horizontal skull plane with stereotaxic frame and measuring skull was then secured with four p and B) Micrometer adjustments of permitted accurate realignment of ter removal of the eye and ear bars and ted the removal of the skull for dissec

Exposure of the semicircular ca measurement required the removal of of the mastoid process and mid structures Each canal was exposed about its curvature from the ampullary to the body of the vestibule After dis each skull was then remounted stereotaxic instrument

To insure proper alignment of the relation to the stereotaxic frame and ing device, both anterior posterior (A- lateral X rays were taken of each skull 1 A and B) Tube to plate distance meters, producing a magnification of 5 X ray tube was aligned by means of a tube with a front to back target separ





;A Lateral X ray of a skull fixed in the stereotaxic ... The temporal bones have been dissected altering ... bony landmarks The measuring device (not ...) is attached to the broad horizontal bar at the ... of the p cture The craniometrically def ned Reid ... (black line in f gure) is defined by black dots placed on the inferior surface of orb ts and at center of external auditory canal Arrows indicate markers made of metal particles placed at the junction of common crus and ves tibule of both labyrinths In this preparation springs sup port the mand ble and a latch holds the convexity of the skull *1 B* Anterior posterior view of skull shown in A



Fig 2 A A right pos view of canal d ssection illustrates the perspecti 2 B and of 3 A B C



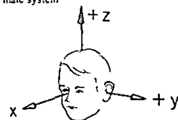
F g 2 B H gher magnif cat of sem c rcular canal d ssect

250 mm Deviations of less than 0 5° from a true lateral and A–P view (at the level of Reid Horizontal Plane) were produced by this alignment procedure

With each skull mounted in the fram planes of excursion of an r ı r manipulator (Prior Farmingdale N Y) aligned parallel to the stereotaxic planes

e I *Mean planar equations for each ~ircular canal plane defined in the Reid otaxic system*

in figure indicates the axes and polarity of the inate system



circular Canal	Planar Equation
~izontal canal	365x 158y 905z 0
erior canal	652x 753y 017z = 0
terior canal	757x + 561y + 320z = 0
orizontal canal	365x + 158y - 905z = 0
nterior canal	652x + 753y 017z = 0
osterior canal	757x 561y + 320z = 0

three dimensional coordinates (to the st 0 1 mm) of a series of approximately ly spaced points along each semicircular were measured by means of a sharp nee- ~tached to the micromanipulator Under ification from 30 to 103 points were ured on each canal, the difference in er being related to differences in canal ter (see Fig 2) Points were taken from ommon crus to the ampulla of each verti- nal and from the body of the vestibule to mpulla in the horizontal canals Data were taken from the medial most por- of the bony vertical canals and from the ior most portion of the bony horizontal Points within the ampullary regions were not included in subsequent calculations

Translational calculations were performed on the original data to refer each point to the stereotaxic zero Each point on a canal thus represented a three-dimensional vector in the Reid stereotaxic coordinate system Data points belonging to each canal were subjected to computer analysis using both a principal component and a multiple regression tech nique (Blanks et al , 1972) Both methods gave the statistically weighted, best fitting planar equation passing through all points for each canal and provided virtually identical results *Only those values derived from principal* component analysis (Thurstone, 1957) will be reported here This technique provides the coefficients A, B and C of the generalized equa tion for a plane $Ax+By+Cz+D=0$ The D term has been set to zero Thus the equations presented are for planes parallel to the nomi nated canal plane passing through the origin This has no effect on the computation of an gles between planes Once derived, planar equations for each canal served as input to several computer programs which provided angular interrelationships between the canal planes

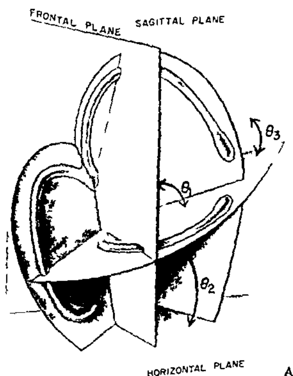
RESULTS

Craniometric measurements on the skull ma- terial revealed a mixture of craniometric shapes The cephalic index (CI) defined as the ratio of the maximum cranial breadth and the

II *Angles in degrees between pairs of human semicircular canal planes*

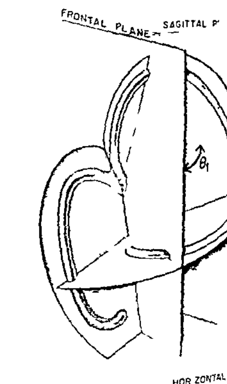
standard deviation (S D) and confidence limits for angles between ipsilateral canal planes represent combined nd left values (N 20) Angles between synergistic canal pairs were considered as unique values and were not ned (N=10)

of canal planes	N	Mean±S D	95% confidence limits Lower	Upper
ontal Anterior	20	111 76±7 55	108 23	115 30
ior Posterior	20	86 16±4 72	83 95	88 38
rior-Horizontal	20	95 75±4 66	93 56	97 93
nterior-Right posterior	10	24 56±7 19	19 41	29 70
osterior Right anterior	10	23 73±6 71	18 93	28 53
orizontal-Right horizontal	10	19 82±14 93	9 14	30.50



ANGLES IN DEGREES BETWEEN HUMAN CANAL PLANES AND THE REID STEREOTAXIC PLANES

Angle between the Horizontal Canal Plane & Stereotaxic		Mean + SD	N	95% Confidence Limits Lower	Upper
Frontal plane	θ_1	68 60 + 5 86	20	65 85	71 34
Horizontal plane	θ_2	25 12 + 5 62	20	22 49	27 75
Sagittal plane	θ_3	99 10 + 8 76	20	95 00	103 20



ANGLES IN DEGREES BETWEEN HUMAN C/ AND THE REID STEREOTAXIC PL/

Angle between the Anterior Canal Plane & Stereotaxic		Mean + SD	N
Frontal plane	θ_1	49 32 + 4 79	20
Horizontal plane	θ_2	89 00 + 4 94	20
Sagittal plane	θ_3	41 10 + 4 82	20

Fig 3 A–C Location of the angles (θ_1 θ_2 θ_3) between the semicircular canal and stereotaxic planes from vector ot product calculations Values represent mean from ned right and left sides (N=20) The right canal planes are illustrated location for values on mirror image locations Angular values an the horizontal canal are shown in A for ant B and for the posterior canal in C

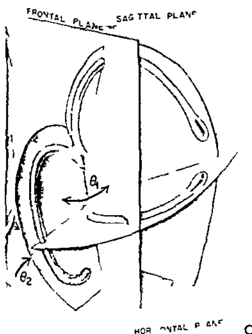
maximum cranial length ranged from 70 to 77 5 Seven skulls were of the dolichocephalic or long skull type (70≤CI≤74 9), and three were of the mesocephalic type (75≤CI≤79 9) (Schaltenbrand & Bailey, 1959)

Planar equations

Planar equations representing the average equation for each canal plane defined in the Reid stereotaxic reference system are given in Table I Equations were derived by averaging the values from each of the ten skulls The insert in Table I depicts the Reid reference axes

The magnitude of the x y and z for each canal is important in pred canal response to roll pitch and ya accelerations respectively It will Table I that each canal has a significa tion into the roll plane (magnitude component) and into the pitc (magnitude of the y component) In component) the horizontal canal (0 the posterior canal (0 320) would be fected by angular acceleration wl anterior canal (0 017) would be virtual fected

While a vectorial representation of th



LES IN DEGREES BETWEEN HUMAN CANAL PLANES AND THE REID STEREOTAXIC PLANES

etween the ior Canal Stereotax c		Mean + SD	N	95% Conf dence L mits Lower	Upper
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tat plane	θ_2	71 36 + 6 20	20	68 45	74 26
plane	θ_3	55 84 + 3 95	20	53 99	57 69

em is useful for kinematic analysis of the m the angular relationships are also help hree sets of angles were calculated (1) formed between pairs of canal planes ose formed between canal planes and the otaxic planes and (3) those which enable ptimal positioning of single canals for ro ial and caloric stimulation

s between pairs of canal planes

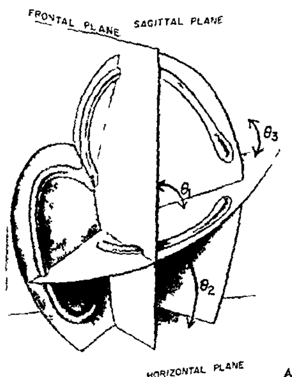
e II shows the calculated angles between ific pairs of canal planes Only small dif ices were found between the correspond anals on the right and left sides and there the left and right data were combined to a population of 20 This was not possible angles between pairs of contralateral synergistically acting canal planes ($n=10$) It will be noted that ipsilateral canal planes do not form true right angles with adjacent canals The more nearly perpendicular pairs were the anterior and posterior canals (86 16°) and the posterior and horizontal canals (95 75°) A much greater degree of non perpendicularity was found between the horizontal and anterior canal planes (111 76°) The fact that 95% con fidence limits on angles between ipsilateral canal planes did not include 90° indicates these canals are not orthogonal

Table II also indicates the degree of co planarity found between pairs of synergistic canal planes Mean angles between the left anterior and right posterior canals and be tween the left posterior and right anterior were 24 56° (S D =7 19) and 23 73° (S D =6 71) Angular deviation between contralateral hori zontal canals was more variable (S D –14 93) but of similar magnitude (19 82°)

Relationship between the canal planes and the stereotaxic planes

The stereotaxic orientation of the right canal planes and the angles made with the stereo taxic planes are given in Figs 3 A B C Figs 2 A and B illustrate the perspective from which Figs 3 A B and C were drawn The right horizontal canal (Fig 3 A) forms an angle of 25 12 with the Reid horizontal plane and is tipped slightly with respect to the sagittal plane (higher medially lower laterally) resulting in a mean angle of 99 10° with the sagittal plane The lateral tilt in both hori zontal canals results in the degree of non coplanarity noted earlier (19 82° between right and left horizontal canal planes) in Table II Most (14/20) horizontal canal pairs showed this lateral inclination while some (?/20) were essentially perpendicular to the sagittal plane and others (4/20) were tipped a few degrees in an opposite fashion (higher laterally lower medially)

The orientation of the vertical canals with respect to the sagittal plane was less variable with the anterior canal forming an angle less



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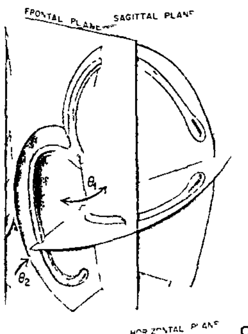
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Table III *Naso-occipital and lateral inclination for each canal plane (defined in text)*
Values for the right and left sides have not been combined to demonstrate degree of right-left symmetry (
'Direction' indicates the orientation of the angle OA=open anteriorly, OP=open posteriorly OL
OR=open right

Semicircular canal	N	Naso-occipital inclination Mean	S D	Direction	Lateral inclination Mean	S D	Direction
Horizontal							
Left	*10*	*21 44*	*4 82*	O A	9 43	9 81	O R
Right	10	22 38	6 95	O A	10 16	9 27	O L
Anterior							
Left	*10*	*91 18*	7 76	O P	91 64	6 77	O L
Right	10	91 31	7 90	O P	91 26	6 75	O R
Posterior							
Left	*10*	*68 01*	7 98	O P	61 00	8 04	O R
Right	10	66 26	7 80	O P	60.52	9 69	O L

than 45° (41 10°) (Fig 3 B) and the posterior canal an angle greater than 45° (55 84°) (Fig 3 C) This general pattern of orientation for the vertical canals in relation to the sagittal plane agrees with the figures given by Colosi & Giannardi (1968) who measured from X ray material the angle formed by the anterior canal (35 5–37°) and the posterior canal plane (59 5–61 5°) in relation to the anterior-posterior axis

Right-left symmetry

To demonstrate the degree of right-left symmetry found in the angular relationships in the present material, a pair of "locating angles" (naso-occipital and lateral inclination angles) are given for each canal which were calculated from the ratio of two directional cosines That is, given a general canal planar equation defined in the Reid stereotaxic coordinate system (Table I) of the form, $ax+by+cz=0$, the naso-occipital inclination is expressed as $\tan^{-1}(c/a)$ and the lateral inclination as $\tan^{-1}(c/b)$ It must be emphasized that these angles represent values projected into a third plane and as such, do not describe the angles between planes as presented earlier (Figs 3 A, B and C, Table II) These angles are given in Table III

As shown in Table III, the naso-occipital inclination of 10 right and 10 left canals was 22 38° and 21 44° The canal lateral inclination values fo canals were 10 16° and 10 left va 9 43° Close examination of the value III for A–P and lateral inclination a cate only small right-left difference values (1–2°) The absence of large values in mirror image canal pairs and left horizontal canal A–P inclin indicates the absence of data skew may have resulted from skull misori the stereotaxic frame

Angular position of the horizontal semi-circular canal with respect to the Frankfurt horizontal plane

In order to compare the results from ent study with the results of others w respect to the Frankfurt plane, it w sary to define the angular relationship the Reid and Frankfurt horizontal Measurements from craniometric an X-rays (Fig 1 A) indicate a small an ference of 4 43±1 14° between the R zontal plane defined by the center of ternal auditory canal and the inferior of the bony orbits and the Frankfurt tal plane defined by the superior mar external auditory canal and the same

s Combining the average right–left val- ·or the horizontal canal naso-occipital in- ·ion in the Reid system (Table III) and the ·ar difference between the two reference ·s (4 43°), the horizontal canals are in- ·1 26 34° with respect to the Frankfurt

ese observations agree closely with the ·nents in the literature that the horizontal ·s are inclined 30° open anterior to the ·ontal plane (Fitzgerald & Hallpike, 1942, ·el & Sommer, 1947) and the anatomical ·urements of 25°±7° by Girard (1923), ·°±4 96° by Fenart & Dardenne (1968), ·–7° by Perez (1922), and about 28°–37° by ·calculation of data of Robin et al (1967) ·what smaller values for the A–P inclina- ·of the horizontal canal with respect to the ·furt plane were obtained by Kudo (1965) ·87′ (sic), by Nishimura (1930) of 12 2°, by ·emann (1904) of 13 03° These smaller ·s may be due to differences in skull shape ·en samples our skulls were classified as ·ocephalic and mesocephalic (3) whereas ·kulls measured by Kudo were brachi- ·alic in shape Further information is ·d to explain this difference

DISCUSSION

lar measurements on the human ·ircular canals in previous studies have ·ed from measurement of X rays (Colosi ·nnardi, 1968, Mincker, 1968) or by direct ·urement of a few points on each canal ·o, 1965, Girard, 1923) Both techniques ·de information on the angular position of ·anals, but the degree of accuracy of these ·iques and the inability to define the canal ·in a stereotaxic reference frame lessens ·alue of these results The method em ·ed in this report, i e , taking a large num ·of approximately equally spaced data ·ts per canal (from 30–103) and then defin ·ach canal with a best fitting plane results ·eater accuracy

rays of each skull in the stereotaxic frame permitted a check on craniometric and X-ray determined skull reference planes Examina tion of X-ray material revealed a small (<2°) misalignment of the Reid planes with the stereotaxic instrument frame in some skulls This was compensated for in the final calcula- tion of all angles noted in the Results

One concern with the present technique, as well as with previously mentioned methods of canal measurement, is that less than the entire 360° circumference of the toroid ring was measured That is, the ring of endolymph which begins in the semicircular canals and is completed through the utricle was not meas ured In most cases the arc of bony canal over which data points were taken was about 174° The degree to which the portion of the en dolymph fluid torus lying within the utricle contributes to the biologically active canal plane is uncertain It can be inferred that if the plane passing through the semicircular canal arc is everywhere continuous with the en dolymph channel within the utricle, then the planes measured here will represent the entire toroid However, if the membranous walls of the utricle distort the endolymph fluid toroid, then some alteration of the functional canal plane would be expected Careful histological examination of the portion of the canal plane within the vestibule will be important in de- termining this possibility

One additional factor in the accuracy of the present technique in determining the position of the biological response plane of the canals is the alignment of the membranous and osseous canals It will be remembered that the present technique measures the medial most portion of the osseous vertical canals and the superior- most portions of the osseous horizontal canal However, the biological plane of the canal is determined by the position of the membranous canal In the human, the membranous canals lie against the walls of the bony canal at the point of maximal osseous canal radius (*R*) (Igarashi, 1966, Curthoys et al , in prepara- tion) This means that the plane of the membranous canal is nearly parallel

Table IV *Positioning angles for human semicircular canal stimulation*

Semicircular canal plane	Rotational				Caloric	
	Pitch angle		Roll angle		Pitch angle	
	Angle	Direction	Angle	Direction	Angle	Direction
Left horizontal canal	21 91	PND	9 11	RRED	68 09	POD
Left anterior canal	91 25	POD	48 90	RLED	1 25	POD
Left posterior canal	67 14	POD	34 16	RRED	22 86	PND
Right horizontal canal	21 91	PND	9 11	RLED	68 09	POD
Right anterior canal	91 25	POD	48 90	RRED	1 25	POD
Right posterior canal	67 14	POD	34 16	RLED	22 86	PND

bony canal plane and would indicate that the angular measurements in the bony canal planes in the present study would apply also to the membranous canal plane In view of the large perilymphatic space in the human canal system, the possibility of a systematic deviation of the membranous canal from the bony canal plane cannot be entirely eliminated The maximal possible angular error in such cases would be related to the ratio of the canal radius of curvature (R) and the difference in cross sectional radii between the osseous (r_o) and membranous (r_m) portions of the canal Among the three canals in the human, biophysical measurements by Igarashi (1966) predict that the largest angular deviation between the os- us and membranous canal planes could occur in the horizontal canal where the combination of a small radius of curvature ($R=3\ 2$ mm) and large perilymphatic space ($r_o-r_m=0\ 63$ mm) result in an angle of $5\ 4°$ ($\tan^{-1}$ [0 63 mm/3 2 mm])

The measurement technique employed here on humans has been shown to be a valid way of establishing semicircular canal planes Planar equations derived by this technique from seven cat skulls (Blanks et al , 1972) have been found to be in close agreement with the physiologically determined response planes of first-order canal neurons in the cat (Estes et al , 1975) Angular differences of from 5–12° were found between anatomically (canal planes when compared with pl cally determined values

Positioning for vestibular stimulation

The most valuable result of the pres is that a mathematical description of system enables the orientation of hu jects for more accurate stimulation vidual canal receptors It should be n because contralateral synergists lel (Table I), orientation of the head f lation of bilateral synergists will be promise each canal will deviate f plane of acceleration Table IV gives angles for each canal which permits p ing of the head for optimal orientatio rotation or caloric stimulation The v this table are calculated using an uprig orientation as an initial position (Reid h tal plane parallel to an earth horizonta order and axis about which the h positioned are important and values calculated on the basis of first pitch the Pitch is rotation about the interaural ax is then defined as rotation about an earth zontal axis coincident with the sagittal Values for rotational stimulation are gi the left columns of Table IV An exam head positioning for stimulation of the

rior canal is a 67° pitch occiput down -)) followed by a 34° roll left ear down 'D)

om the results of this study the optimal ion for stimulation of both horizontal s is by an angular acceleration in an earth ontal plane with the head pitched forward ose down from the Reid horizontal plane above) Because of the noncoplanarity of horizontal canals in this position each ontal canal is approximately 9° out of the of stimulation Also in this position the or canals form angles of 75° with the of stimulation whereas the posterior s are almost exactly perpendicular to the of stimulation (90 8°) Because the ior canals are not perpendicular to the of the acceleration, they will both be lated by an angular acceleration about hird the magnitude of that applied to the ontal canals (cos [75]=0 26)

o given in Table IV are figures for posi g the subject for caloric stimulation , calculations were based upon an initial nt head *position A single rotation of* 68° for the horizontal canal, 10° POD for the or canal, and 23° POD for the posterior is required to bring the ' target canal in earth vertical position It should be that the last two positionings still leave orizontal canal angulated from earth hori

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ZUSAMMENFASSUNG

pal-component Analysen wurden an einer Serie von von Reid berechnet Die Resultate zeigen dass Paare von ipsilateralen Kanalen nahezu rechtwinklig zueinander stehen Eine Ausnahme bildet der Winkel zwischen dem *vorderen und horizontalen Kanal* (*Mittel=111°*) Synergistische Kanalpaare bilden einen Winkel von 19° zwischen dem rechten und linken horizontalen Kanal und von 23-24° zwischen vertikalen Paaren Der horizontale Kanal bildet einen Winkel von 25° mit der Reidschen Horizontalebene Mittels mathematischer Gleichungen wurde die optimale Kopfposition für rotatorische und kalorische Reizung ermittelt.

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C H Markham M D
Dept of Neurology
University of California School of Medicine
Los Angeles Calif 90024, USA

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THE ORIENTATION OF THE SEMICIRCULAR CANALS IN THE GUINEA PIG

I S Curthoys,[1] E J Curthoys,[2] R H I Blanks[3] and C H Markham[4]

From the Department of Psychology University of Sydney Sydney N S W Australia[1] [2] and the Department of Neurology Medical School University of California Los Angeles Calif USA[3] [4]

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...t In 10 adult guinea pigs the stereotaxic coordi... f a series of points along each osseous semicircular ...were analysed to yield an equation of that canal ...n stereotaxic space Angular relationships among ...al planes and between the canal planes and the ...tereotaxic planes are presented together with the positions of the head for physiological stimulation ...canal or pair of synergistic canals The planes ...micircular canals in each labyrinth are not perpen... to one another and the planes of contralateral ...stic canals depart from parallelism by about 30°

...ler to specify the mechanical forces in... ...d in semicircular canal stimulation it is ...sary to have information about, among ...things, the fine structure of the canal and ...rientation of the canal relative to the ... of the stimulating acceleration Optimal ...ological stimulation occurs when the ...plane is parallel to the plane of rotation ... canal is tilted from parallelism the ...itude of the angular acceleration stimula- ...decreases as a cosine function (Blanks, ... van Egmond et al , 1952) and other ca... ...are increasingly stimulated We have de... ...ed a technique which determines the orientation of the semicircular canal planes with reference to external skull landmarks (Blanks et al , 1972, 1975) enabling the head to be positioned to optimally stimulate any canal This technique has yielded data for the cat and human the present paper reports comparable information for the guinea pig

The guinea pig has been used extensively in anatomical and physiological studies of the vestibular system Its semicircular canals are about the same size as those of the cat and much more easily accessible (Curthoys et al , 1971) Nevertheless there is surprisingly little data concerning the orientation of these canals in the skull Kristensen (1954) reported that the planes of the horizontal canals are parallel with a plane passing through the external auditory meati and the supraorbital margins Wersall (1956) reported that the two horizontal canals are in the same plane and the vertical canals are perpendicular to this plane He estimated the angle between the ipsilateral anterior and posterior canal planes to be 100°, Bodechtel (1930) has estimated this angle to be 115° Wersall also reported that the two posterior canals formed an angle of 110° with each other These results lead to the deduction that both pairs of vertical synergistic canals (the left anterior right posterior and right anterior-

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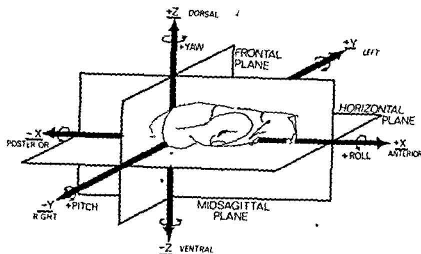


Fig 1 The stereotaxic guinea pig and the ma ventions used

left posterior) are misaligned from parallelism by about 25°

Because of the need for a more complete and accurate specification of semicircular canal planes in the guinea pig, particularly for skull positioning during angular acceleration stimulation we applied a technique, described in detail elsewhere (Blanks et al , 1972) to determine these planes in the stereotaxic axes system

METHODS

Guinea pig stereotaxic planes

In stereotaxic systems the horizontal plane is the most difficult to define for the cat, monkey and human, something akin to the Reid plane is used (the plane joining the centres of the external auditory canals and the inferior margins of the orbits) Definitions of the stereotaxic horizontal plane for rodents are far less uniform (de Groot, 1959, Hurt et al , 1971, Konig & Klippel, 1963, Luparello, 1967, Luparello et al , 1964, Pellegrino & Cushman, 1967, 1971, Tindal, 1965) Tindal did define the horizontal plane for the guinea pig by the external auditory canals and the inferior margin of medial canthi However, his animals were so unusually large (700–830 g) that he was able to use a Kopf cat/monkey head holder The other widely used stereotaxic system for the guinea pig defines the horizontal plane by means of the heights of the skull surf and 14 mm anterior to the intera (Luparello, 1967) Instead of using e these systems we chose to use the Kopf pig head holder with the incisor vert justment set at zero In this way the hr tal plane becomes the plane throug centres of the two external auditory can the inferior-most portion of the bone pos erior to the incisors (i e the rostral edge incisor bar) Measurements of a num skull dimensions enabled simple transl the semicircular canal planes to the s outlined above The sagittal plane s pendicular to the horizontal plane p through the midsagittal suture The plane is perpendicular to the two others passing through the centres of the ex auditory meati The Hixson, Niven and reia mathematical conventions wer (1966) in this system the *x* axis is the n cipital axis (anterior positive) the y ax interaural axis (left ear positive) and the is the vertical axis (dorsal positive) (see 1 for these conventions)

Dissection and measurement

Eleven adult guinea pigs with weights ra from 311 to 487 g (mean 381 g) were k barbiturate overdose and the tissue re from their skulls Dental cement was to the skull sutures to prevent disloc



An overview of a guinea pig skull from a right ... point posterior to the interaural axis to show the ... of Fig 3 and Fig 4 A B, C

...kulls were dried and for each animal the ...us canal of every semicircular canal was ...d along as much of its length as pos... by drilling An attempt was made to ...ardize this dissection, so that for all ...ds the lateral-most bone in each canal ...emoved (see Figs 2 and 3) The bony ...al auditory meati and most of the bullae ... left intact, permitting the skull to be ...ted in a Kopf guinea pig stereotaxic head ...r (Model 1216) in fairly normal fashion ...lard guinea pig ear-bars were used and ...eight of the incisor bar was set at 0 Thus ...ositioning of the skull in the stereotaxic ...ratus was the same as that which would ...ed with a living guinea pig

Kopf electrode carriage with micro... ...ipulator controls (model 1260) was placed ...he stereotaxic frame and its planes of ...ursion were aligned with the stereotaxic ...es About 50 mm of fine (80 μm diameter) ...nless steel wire insulated except at the tip ... affixed to the electrode holder and con... ...ed to a voltage follower, audio amplifier ... loudspeaker Touching the tip of this wire to an osseous canal or reference point resulted in a loud "pop" which considerably assisted identifying the "touch point" The reference point consisted of a short piece of 80 μm wire cemented to the top of a bolt The cement was filed so the tip of the wire was flush with the surface This reference point was bolted to the stereotaxic frame and it remained untouched during all measurements

Each skull was placed in the stereotaxic de vice and its position was not changed during measurement The x, y and z coordinates of a series of approximately equally spaced points along each canal were obtained by advancing the wire probe until it just touched the medial most wall of the osseous canal and reading the scales to the nearest 0 05 mm Points in the ampullary and common crus reg ions were avoided An average of 28 points per canal were recorded (range 17 to 50) After measuring the canals in one labyrinth a check was made to ensure the probe had not been displaced and if this check was satisfactory the probe was repositioned to enable readings of the contralateral labyrinth The coordinates of



Fig 3 An enlarged port on of Fig 2 T shows the osseous semicircular canals are shown schem Fig 4 A B C

points along a semicircular canal were always referred to the reference point which was in turn referred to the stereotaxic zero (the point of intersection of the three planes defined above) thus relating all canal measurements to stereotaxic zero

RESULTS

Statistical treatment

For each skull the raw data consisted of the x y and z coordinates of points along each canal This data was punched onto cards and the coordinates translated into stereotaxic space by subtraction This data was plotted printer to ensure there were no pun rors outlying data points or substa between data points along any canal T ter occurred for the anterior canals of animals because of breakage during dis of the extremely thin part of the anterior near the common crus (see Fig 2) The als were excluded from all further a One animal was excluded entirely beca tailed analysis showed it had not positioned properly in the sterotaxic d plane was fitted to the data for ea h ca least squares technique and by print

·I Equations of the planes parallel to the ırcular canals passing through the ɔtaxic zero

gure ın brackets below each coefficient ıs the rd deviation

ɔnzontal	+ 716x	− 263y	− 637z	=0
	(± 008)	(± 0'0)	(± 011)	
ıtenor	−578x	+ 793y	− 167z	=0
	(± 013)	(± 008)	(± 018)	
ɔstenor	− 771x	− 380y	−.503z	=0
	(± 011)	(+ 014)	(± 010)	
onzontal	+ 716x	+ 263y	− 637z	−0
	(± 008)	(± 020)	(± 011)	
ntenor	+ 578x	+ 793y	+ 167z	=0
	(± 013)	(+ 008)	(± 018)	
ostenor	− 771x	+ 380y	− 503	=0
	(± 011)	(± 014)	(± 010)	

ıt analysıs As ın earlıer studıes both ques gave vırtually ıdentıcal results but .he results of the prıncıpal component ıs are reported here Thıs technıque the dırectıon cosınes of a plane passıng :h the orıgın parallel to the plane of best the data poınts of a gıven canal (Thurs- 1957) In other words the D term of the ılized equatıon of a plane ın three dımensıons $Ax+By+Cz+D=0$ has been set to zero (Bers, 1969) The equatıons of the plane parallel to each canal are presented ın Table I The coeffıcıents ın these equatıons are the mean dırectıon cosınes Sınce magnıtude dıfferences between dırectıon cosınes from left and rıght labyrınths were small, they were combıned to ımprove estımatıon Thıs average value was then gıven the correct sıgn for the approprıate labyrınth

For each anımal the dırectıon cosınes of ıts canal planes served as ınput for another program whıch computed the angles among the canal planes and between each canal plane and the stereotaxıc planes Magnıtude dıfferences for angles from left and rıght labyrınths were small and hence these angles were combıned wherever possıble to ımprove estımatıon These mean angles are presented ın Table II together wıth the numbers upon whıch each mean ıs based, the standard devıatıons and two-taıled 95% confıdence ıntervals (Guenther, 1965) Fıgs 2 and 3 show the orıentatıon of the canals ın a guınea pıg skull and provıde the perspectıve for Fıg 4 A, B, and C

ᵼ II *Means number of measurements (n) standard devıatıons and 95% confıdence lımıts ıgles ın degrees, among ıpsılateral canal planes between contralateral synergıstıc canal ɟ and between each canal plane and the stereotaxıc planes The locatıons of these last set ıles are shown ın Fıg 4 (see Column 1)*

ɔn and catıon	Angle between the plane of the		Mean	n	S D	95% confıdence lımıts Lower	Upper
	Horızontal canal	Anterıor canal	122 15	17	6 13	118 99	125 30
	Anterıor canal	Posterıor canal	76 71	17	5 49	73 89	79 53
	Horızontal canal	Posterıor canal	82 36	20	4 74	80 14	84 58
	Left horızontal canal	Rıght horızontal canal	30 82	10	10 05	23 63	38 01
	Left anterıor canal	Rıght posterıor canal	3? 17	8	4 42	28 47	35 87
	Left posterıor canal	Rıght anterıor canal	36 16	9	4 86	30 81	41 51
A θ_1	Horızontal canal	Stereotaxıc frontal	44 17	?0	2 98	42 78	45 57
A θ_2	Horızontal canal	Stereotaxıc horızontal	50 31	20	3 80	48 53	52 09
A θ_3	Horızontal canal	Stereotaxıc sagıttal	105 34	20	5 43	102 80	107 88
B θ_1	Anterıor canal	Stereotaxıc frontal	54 71	17	3 82	52 75	56 67
B θ_2	Anterıor canal	Stereotaxıc horızontal	99 78	17	4 36	97 54	102 02
B θ_3	Anterıor canal	Stereotaxıc sagıttal	37 36	17	3 24	35 69	39 03
C θ_1	Posterıor canal	Stereotaxıc frontal	39 34	?0	4 39	37 29	41 40
C θ_2	Posterıor canal	Stereotaxıc horızontal	59 77	20	2 84	58 44	61 10
C θ_3	Posterıor canal	Stereotaxıc sagıttal	67 60	20	3 99	65 74	69 47

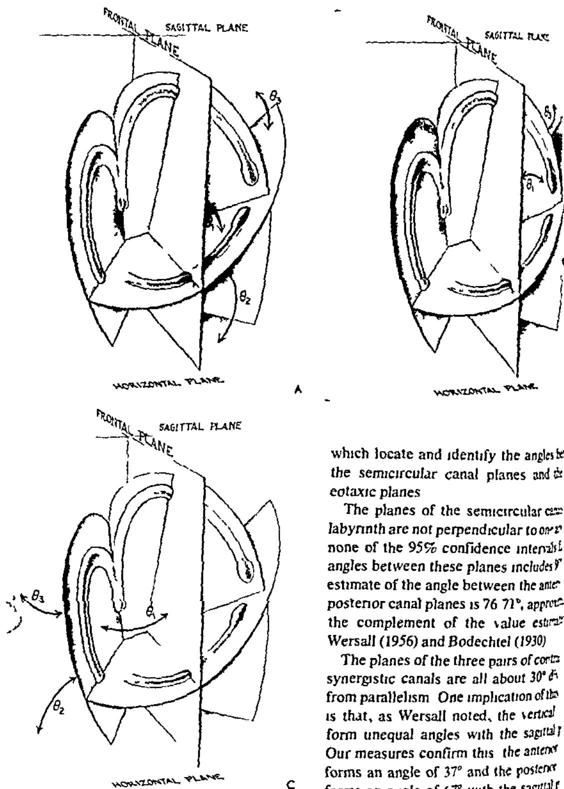


Fig 4 A B C The intersection of the semicircular canal planes on the right side of the head with the guinea pig stereotaxic planes θ_1 θ_2 θ_3 identify the angles between the canal plane and each stereotaxic plane the magnitudes of which are given in Table II

which locate and identify the angles be the semicircular canal planes and the eotaxic planes

The planes of the semicircular can labyrinth are not perpendicular to one an none of the 95% confidence intervals angles between these planes includes 9 estimate of the angle between the anter posterior canal planes is 76 71°, approx the complement of the value estima Wersall (1956) and Bodechtel (1930)

The planes of the three pairs of contra synergistic canals are all about 30° d from parallelism One implication of this is that, as Wersall noted, the vertical form unequal angles with the sagittal Our measures confirm this the anterior forms an angle of 37° and the posterior forms an angle of 67° with the sagittal

Each horizontal canal forms an a 50 31° (open anteriorly) with the hor stereotaxic plane and is inclined (lower b ly) from the stereotaxic sagittal pl 105 34° The vertical canals are no

III *Magnitude of pitch and roll man-es in degrees and direction of rotation ed to bring any semicircular canal into rth horizontal plane of rotation*

pitch nose down PTD–pitch tail down PRED ight ear down RLED=roll left ear down

	Pitch	Direction	Roll	Direction
nzontal	48 37	PND	15 34	RRED
tenor	73 48	PTD	52 64	RLED
stenor	56 83	PTD	22 40	RRED
onzontal	48 37	PND	15 34	RLED
ntenor	73 48	PTD	52 64	RRED
ostenor	56 83	PTD	22 40	RLED

ular to the stereotaxic horizontal plane nterior canal forms an angle of 99 78° he posterior canal an angle of 59 77° the horizontal plane This nonper-ularity would have resulted in errors al plane angles had been estimated by ndez & Valentinuzzi's (1968) projection d

the purpose of positioning a guinea pig gular acceleration stimulation the pitch ll angles of each canal are needed The angle is that angle formed between the intersection of the canal plane and the l plane measured along the nasooccipi-s After pitching the animal by the re- amount the roll angle is the angle between the interaural axis and the line rsection between an earth vertical plane ndicular to the sagittal plane and the plane The pitch and roll angles for each and the direction of rotation for ological stimulation by an earth horizon ne of rotation are given in Table III In lly positioning the animal the rotations ot independent (Goldstein, 1965) and the of the manoeuvres must be pitch then The manoeuvres start from the skull be the standard stereotaxic position shown g 1 So for example to bring the right ior canal into an (earth horizontal) plane tation requires pitching the head 73 48° lown and then rolling the animal 52 64° down

Translation to other stereotaxic systems

Luparello (1967) specified that when the heights of the two points on the skull surface 6 mm and 14 mm anterior to the interaural axis were identical the guinea pig skull was horizontal In our stereotaxic system these points were an average of 1 10 mm discrepant (±0 50 standard deviation) These points would be at the same height if the skull were pitched nose down by 7 38° Thus to translate the positioning angles presented above into Luparello's system requires a pitch nose down of 7 38° the pitch angle of the horizontal canals becomes 40 54° open anterior

The inferior margins of the medial canthi were on average 21 79° above (±1 35) the stereotaxic horizontal plane Translation to Tindal's stereotaxic system requires a pitch nose down by this amount the pitch angle of the horizontal canals becomes 26 58° open anterior

In some definitions of stereotaxic horizontal for rodents the incisor bar is set 2 5 mm below the external auditory meatus (Hurt et al, 1971) In our skulls this corresponds to a pitch of 3 34° (±0 12) nose down

The junction of the coronal suture and the superior orbital ridge was 40 13° above (±1 51) the stereotaxic horizontal plane This point was measured as being the most probable to lie on Kristensen s line between the external meatus and the superior margin of the orbit As an estimator of the pitch of the horizontal canal Kristensen's line probably underestimates the true pitch by about 8° (48 37°–40 13°=8 24°)

DISCUSSION

It is important to emphasize that the planes of the semicircular canals given in this paper are planes derived from measurement of the osseous semicircular canals The extent to which these planes correspond to the planes of the membranous ducts depends on the width of the perilymphatic space A A Gray (1907) and O Gray (1951) have noted that the

perilymphatic space of rodents is usually very small or absent This fact can be verified by a photograph of a decalcified guinea pig labyrinth in O Gray (1955) and we have also confirmed it by measurements of guinea pig membranous labyrinths fixed in 10% formalin Since the radius of curvature of the guinea pig canals is of the order of 1 90 mm (Jones & Spells, 1963, Curthoys et al , 1975 *a*) and the perilymphatic space is of the order of 0 02 mm, it follows that any deviation between the plane of the osseous canal and that of the membranous duct would be about 1° (arctan (0 02/ 1 90))

It should also be stressed that the statistical techniques we used provide a best fitting plane to the series of data points for a canal It was apparent from insepction of the dissected osseous semicircular canals that there are systematic departures from a plane, particularly for the anterior and posterior canals Nevertheless these departures are not large and for the purposes of this investigation it was deemed adequate to use a linear approximation This issue of the planarity of semicircular canals will be considered in more detail in a forthcoming paper (Curthoys et al , 1975 *a*)

One of the major aims of this study was to establish the optimal positioning of the guinea pig skull for physiological stimulation of a given set of semicircular canals and to determine the extent to which other canals would be stimulated when the head was in this optimal position It is clear from Tables II and III that it is impossible to position any two canals to be parallel to a single plane of stimulation The optimal position for bilateral stimulation of the horizontal canals is with the skull tipped 48° nose down relative to an earth horizontal plane of stimulation In this position each horizontal canal is approximately 15° out of the plane of stimulation, the anterior canals both form an angle of 71 19° and the posterior canals both form an angle of 75 91° with the plane of stimulation Thus when the guinea pig head is optimally positioned for stimulation of both horizontal canals the vertical ca[...] receive an appreciable proportion [...] stimulating angular acceleration For [...] if a guinea pig were positioned for stimulation of both horizontal canals a[...] an angular acceleration of 4°/sec² t[...] anterior canals would both receive an [...] acceleration of 1 29°/sec² (cos 71 91°[...] the posterior canals an angular acce[...] of 0 97°/sec² (cos 75 91×4) These [...] levels of the vertical canals would be [...] to influence the firing of sensitive pri[...] secondary vertical canal neurons [...] 1973) and could lead to misunders[...] and misinterpretation in studies of [...] convergence (Curthoys et al , 1971 [...] 1973, Markham & Curthoys 1972)

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We are grateful to Miss Gwynne Gloege for [...] tions Mr N Simpson for photography Dr Ma[...] and Dr Peter Wenderoth for statistical adv[...] Lorraine Nash for typing

ZUSAMMENFASSUNG

Bei 10 ausgewachsenen Meerschweinchen [...] stereotaktische Koordinaten von einer Reihe [...] an jedem knöchernen Bodenganges entlang [...] um eine mathematische Gleichung für den [...] Bodengang im stereotaktischen Raum zu erhalten [...] elbeziehungen innerhalb der Gehorgange und [...] den Gehörgängen werden angegeben sowie [...] lungen des Kopfes fur den physiologischen [...] Gangs oder jedes Paars von synergistischen [...] Ebenen der Bodengange in jedem Labyrinth [...] senkrecht zueinander und die Ebenen der kon[...] synergistischen Gange weichen von der Parallel[...] Grad ab

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I S Curthoys Ph D
Dept of Psychology
University of Sydney
Sydney N S W
Australia

Acta Otolaryngol 80 206-213, 1975

INFLUENCE OF THE HYPOTHALAMIC STIMULATION ON VESTIBULAR NUCLEI UNITS IN THE RAT

T Matsunaga and T Kubo

From the Department of Otolaryngology Kansai Rosai Hospital Amagasaki City Japan

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Abstract Electrical stimulation of rat hypothalamus in fluenced the activity of the vestibular nuclei units de *pending on the stimulus location* Vestibular units were excited predominantly by antero-lateral hypothalamic

In an earlier paper (Kubo et al 1[illegible]) reported that the electrical stimulatio[illegible] vestibular nuclei induced the evo[illegible] *sponses in the hypothalamus bilateral*[illegible] paper is intended to demonstrate a des[illegible] influence from the hypothalamus to [illegible] tibular nuclei

It is generally considered that many factors such as disturbances of the endocrine system autonomic nervous system and psychiatric stress are closely related to the inducement of vertigo or dizziness (Wodak, 1959) However, it is not clear how or why these factors cause this complaint It has earlier been clearly de monstrated that there is close interaction be tween the peripheral autonomic system and vestibular system (Watanabe 1967) At pres ent, the role of the central autonomic nervous system in vertigo and/or dizziness especially where the hypothalamus is concerned is still obscure Clinical experience suggests that the hypothalamus does play a part in the incidence and development of this disorder (Spencer Harrison & Naftalin 1968 Wood 1968 Egami 1972 Naito 1973 Hinoki et al 1973) It therefore seems very necessary to clarify the role of the hypothalamus in vertigo

Prompted by the above mentioned consid erations the correlation between hypo thalamus and vestibular nuclei has been in vestigated

MATERIALS AND METHO[illegible]

Vestibular units response

The experiments were conducted on [illegible] albino rats Surgical and recording m[illegible] were identical with those reported in t[illegible] vious paper (Kubo et al 1974) The [illegible] was placed in a stereotaxic frame on th[illegible] tion table, which moves sinusoidally [illegible] of 3~10 sec and total amplitude of [illegible] head was inclined downwards as much a[illegible] sible due to the horizontal location of [illegible] eral semicircular canal and fixed at the [illegible] of rotation

For the electrical stimulation two st[illegible] steel concentric bipolar electrodes w[illegible] meters of 0 2 mm and intertip dist[illegible] 0 5 mm were inserted through a small [illegible] trephine and an intact cortex to the [illegible] thalamus with the aid of the stereotax[illegible] atlas (Pellegrino & Cushman 1967) electrode was aimed at the lateral [illegible] thalamus (level head co-ordinates 0.5-[illegible] behind bregma 1 5 mm lateral to sagi[illegible]

Effects of hypothalamic stimulation ·bular neurons

	I	II	III	Total
: to				
	24	33	1	58
nse				
ıc				
ıon	17	38	0	55
: to				
ıon	31	28	2	61
	14	17	0	31
	17	11	2	30

ıd 7~9 mm beneath dura) and medial ıalamus (2~3 mm behind, 0 5 mm lateral ·9 mm depth)

recording the vestibular unitary re ·, the glass microelectrode, with a tip ·er of 1~5 μm and filled with lithium ıe, was inserted stereotaxically through l occipital trephine and intact cerebel the vestibular nuclei When a unit was ·ntered its response to rotation was

effect of hypothalamic stimulation on those identifiable neurons which re ·d to sinusoidal rotational stimulus, was ıned An electrical stimulus was applied rly through the posteromedial and lateral electrodes in the hypothalamus l

tangular pulses were delivered with the ıng parameters 30/sec, 0 2 msec, 5~10 l maintained for 5~10 seconds

ated evoked field potential

h series of the above mentioned experi an attempt was made to identify the ated field potentials within the vestibular by stimulation of the hypothalamus

his case, single pulse stimulus was used volts, 0 2~0 5 msec, 2~3/sec)

: evoked potentials were picked up polarly between the glassmicro- ode and the reference electrode in the and were multiplied 30 times by the computer and photographed with a polaroid camera

The sites of the hypothalamic stimulation were determined by making an electrolytic le sion at the tip of the electrode The recording electrode tip position was established by the development of red spots using the technique of Mitarai (1960)

RESULTS

Response to angular stimuli

Fifty eight vestibular neurons were identified and classified by their response to sinusoidal rotational movement (Shimazu & Precht, 1965) Twenty four were type I, 33 type II, and I type III (Table I)

Fig 1 shows the original action potentials from a single cell located in the left medial vestibular nucleus during sinusoidal rotation at a frequency of 0 2 Hz The upper illustrated curve indicates the position of the turntable (up–left going)

Response to hypothalamic stimulation

The hypothalamus was systematically stimu lated in all of the hypothalamic areas which are medial to the fornix and extend from the rostral portion of the mamillar body in the infundibular region of the ventromedial hypo- thalamic nucleus and lateral to the fornix and from the rostral portion of the infundibular region to the chiasma optica

Two areas in particular responded to hypo thalamic stimulation These were the anterior hypothalamus in the lateral hypothalamic area of the infundibular region (AH), and the mid dle hypothalamus in the medial hypothalamic area of the same region (MH) which is almost at the level of the ventromedial nucleus In all examined animals it was histologically ver- ified that the anterolateral electrode track was located in the AH and the posteromedial electrode track was positioned in the MH

Fig 2 demonstrates the facilitatory response

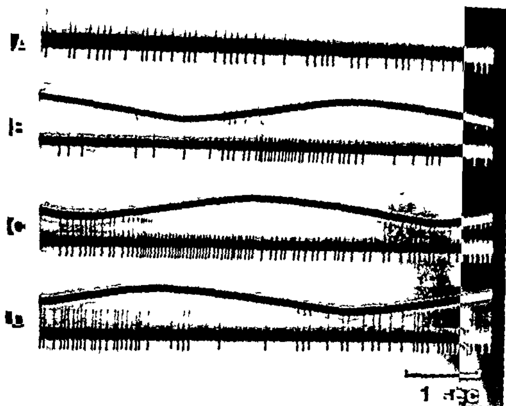


Fig 1 Original records of sinusoidal rotation (period 5 sec total amplitude 110°) and original AP train *A* indicates the spontaneous discharge in Type 1 neuron in the left vestibular nuclei In *B C D* the upward angular displacement indicates leftward ment

of the spontaneous discharge in a vestibular neuron to the electrical stimulation of the AH

Fig 3 shows the inhibitory response of the spontaneous discharge in another vestibular neuron to the stimulation of the MH

Each vestibular neuron was subjected to two kinds of stimulation, AH and MH, so that while 58 neurons were examined in this experiment, the responses elicited totalled twice this figure, i e 116 Thus, of the 58 neurons included in the analysis, 61 positive responses were evoked by hypothalamic stimulation, leaving a negative response total of 55 Of the 61 positive responses, 31 responded with facilitation, and the remaining 30 responded with inhibition Thus, about half the neurons responding to sinusoidal rotation responded to hypothalamic stimulation with a corresponding degree of facilitation or inhibition

Of 24 type I neurons 65% (31 positive responses out of 48) responded to hypo stimulation In contrast, in 33 type II the positive response totalled 44% (29 responses out of 66) (Table I)

It seems that the type I neuron is m ily influenced by hypothalamic st than the type II neuron On the oth there was no significant difference in portion of positive responses eve hypothalamic stimulation with either tion or facilitation in the group of neurons, as compared with the group of neurons

Summated evoked field potential

Prompted by the above mentioned find the responses of the vestibular activit electrical stimulation of AH and MH n some differentiation an attempt was identify evoked field potentials within t

[10] Effects of stimulation of the anterior hypothalamus [illegible] lateral hypothalamic area (*AH*) upon spontaneous [illegible]rge of the ipsilateral vestibular neuron (Type II) *A* [illegible]neous discharge *B* onset of stimulation *C* during stimulation *D* immediately after end of stimulation The short downward line indicates the artifact of stimulation (6 V 0 ? msec 30 Hz) *E* temporary inhibition after stimulation

[illegible]ar nuclei by a single pulse stimulus of the [illegible]thalamus All neurons which responded [illegible]nusoidal rotatory movement failed to re[illegible]d to a single pulse stimulus of the hypo[illegible]mus However, we were able to record [illegible]ummated evoked field potential in the ves[illegible]ar nuclei in MH as well as in AH (Fig 4) [illegible]ther words the electrical stimulation in [illegible] produced the evoked potential with a [illegible]ophasic negative wave of about 15~20 [illegible] peak latency

[illegible]hile the electrical stimulation in AH also [illegible]luced a monophasic negative evoked po[illegible]tal of the same latency, it sometimes had a tendency to elicit a monophasic positive wave (Fig 4)

The phase reversal (turn over point) of the summated evoked potential was not recorded and the amplitude was also of the same order in cerebellum and reticular formation Following these results the relation between the development of the evoked potential in the vestibular nuclei and the response of spontaneously active vestibular neurons by the electrical stimulation of AH and MH was examined Table II sets out the summarized results In positive cases of the summated evoked potential 10 of the 27 neurons in AH stimulation

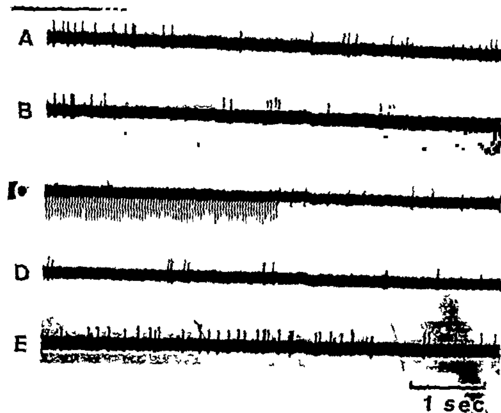


Fig 3 Effects of stimulation of the middle hypothalamus in the medial hypothalamic area (MH) upon spontaneous discharge of the other ipsilateral vestibular neuron (type II) *A* spontaneous discharge *B* onset of stimulation *C* immediately after end of stimulation The dots indicates the artifact of stimulation (6 V 0.2 msec) *D* temporary inhibition after stimulation *E* facilitation

responded with a facilitation of spontaneous activity, 6 with inhibition and 11 with no response In cases of the non evoked potential, 6 of the remaining 31 exhibited facilitation 7 inhibition and 18 no change of spontaneous discharge in AH stimulation

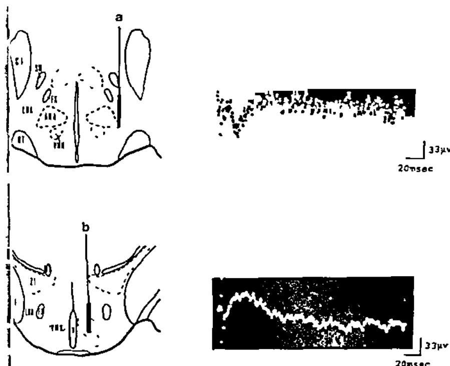
In MH stimulation, 6 of the 28 neurons in the presence of the evoked potential responded with a facilitation of spontaneous activity, 12 with inhibition and 10 with no response However, of the remaining 30 neurons in cases of non-evoked potential, 9 exhibited facilitation, 5 inhibition and 16 no change of spontaneous discharge in MH stimulation Thus, the AH stimulation is apt to facilitate more easily the spontaneous discharge in the vestibular neurons, and the MH stimulation seems to inhibit more easily the spontaneous discharge, when the evoked response is recorded in AH and MH stimulation

DISCUSSION

Generally speaking, there seem to [illegible] schools of thought concerning the fu[illegible] the autonomic nervous system in the [illegible] thalamus

One is that the lateral hypothala[illegible] belongs to the parasympathetic divis[illegible] that the medial hypothalamic area [illegible] the sympathetic division Yuasa (19[illegible] ported that the electrical stimulation [illegible] lateral hypothalamic area increased the spontaneous discharge and also that the stimulation of the medial hypothala[illegible] increased the cervical sympathetic d[illegible] in rabbits

On the other hand according to Ge[illegible] (1967) the anterior hypothalamic area [illegible] utes to the parasympathetic effects a[illegible] posterior hypothalamic area to the sy[illegible] tic effects



Schematic drawing of the hypothalamus and the ...ating sites (left column) and summated evoked field ...als in vestibular nucleus (right column) Left col ... indicates electrode track positioned at the anterior ...alamus in lateral hypothalamic area (*AH*) and *b* ...es electrode track positioned at the middle hypo...us in medial hypothalamic area (*MH*) Right col ...pper picture positive evoked potential from the stimulation of AH (3 V 0 5 msec 3 Hz) average of 30 cycles Lower picture negative evoked potential from the stimulation of MH (3 V 0 5 msec 3 Hz) Abbreviations *AHA* anterior hypothalamic area *CI* capsula interna *FX* fornix *LHA* lateral hypothalamic area *OT* optic tract *SM* stria medullaris thalami *V* ventriculus *VMH* ventromedial nucleus of hypothalamus *ZI* zona incerta

...e results of our experiments have demon...d that the activity of vestibular neurons ...nding to the sinusoidal rotatory move... is altered in two ways, facilitation and ...ition, by the electrical stimulation of cer... areas of the hypothalamus Furthermore, ...wo sorts of summated evoked potentials ...e vestibular nuclei, monophasic negative ... positive were elicited by the repeated ...e pulse stimuli of some areas of the ...thalamus Interestingly, in AH stimula... the activity of vestibular neurons re...ded predominantly with a facilitation in ...tive cases of monophasic-negative and ...itive evoked potential, and in MH stimula... it responded mainly with an inhibition in ...tive cases of monophasic positive evoked ...ntial Concerning the descending influence from the forebrain to the vestibular neurons, Arslan & Molinari (1965) reported that the vestibular neuronal activity was modulated in terms of facilitation or inhibition by the electrical stimulation in certain areas of the cortex According to Gildenberg & Hassler (1971) most vestibular neurons were facilitated by the cortical stimulation

It has been shown by Cooper & Rolls (1974) that units in the pons and medulla are activated by the self-stimulation of the lateral hypothalamic region and sites along the medial forebrain bundle in the rat

From anatomical data regarding hypothalamic fiber connection it is well known that the descending efferent pathways of the hypothalamus are contained mainly in the dorsal longitudinal fasciculus and the medial

Table II
( ) positive wave

	Facilit	Inhibit	No response	Total
Hypothalamic stimulation (ant hypothalamus)				
Evoked response (+)	10 (3)	6 (2)	11 (3)	27 (8)
Evoked response (−)	6	7	18	31
Total	16 (3)	13 (2)	29 (3)	58 (8)
Hypothalamic stimulation (mid hypothalamus)				
Evoked response (+)	6	12	10	28
Evoked response (−)	9	5	16	30
Total	15	17	26	58

forebrain bundle (Nauta & Haymaker 1969) *Ban (1964, 1966) reported that there is a* polysynaptic neural circuit between the medial hypothalamic area and the vestibular nuclei via the dorsal longitudinal fasciculus, as we have verified electrophysiologically (Kubo et al, 1974) Thomas & Calaresu (1974) stated that the electrical stimulation of the posteromedial hypothalamus inhibited the vagal *bradycardia elicited by stimulation of localized* medullary region and that it was probably mediated by the dorsal longitudinal fasciculus from the existence of the two sorts of biphasic field potentials

Therefore, the difference in the response of vestibular neurons and in the polarity of the summated evoked potential to the hypothalamic stimulation appears to depend on the difference in the stimulation site of the hypothalamus and in the descending neural circuit from the hypothalamus to the pons and medulla including the vestibular nuclei

We confidently suggest that the electrical stimulation of the central sympathetic division inhibits the spontaneous discharge in the vestibular neurons and the same stimulation of the central parasympathetic division facilitates the spontaneous discharge

Although we do not completely deny the electrical spread in such a stimulation of the hypothalamus there seems to be no doubt that there are differing responses to the [illegible] *lation of such* a circumscribed [illegible] the hypothalamus, as recent evide[illegible] come to light that a set of neur[illegible] which, when activated, elicit an [illegible] functional response in this area (O[illegible] 1964)

It is therefore quite probable that [illegible] tibular neuronal activity will be mod[illegible] the hypothalamic descending influence [illegible] produces two different effects [illegible] sympathetic or parasympathetic [illegible] *through the medial forebrain bundle* [illegible] dorsal longitudinal fasciculus

However, the question arises as to [illegible] the modulation of the vestibular [illegible] activity is induced only by the aforem[illegible] *neural effect from the hypothala[illegible]* vestibular nuclei, as it has been know[illegible] hypothalamic stimulation induced [illegible] secretions of noradrenalin and adre[illegible] varying proportions depending on the [illegible] tion of the stimulus in cats (Folko[illegible] 1954) This excreted noradrenalin an[illegible] lin may possibly modulate the [illegible] *neuron activity (Yamamoto 1967)* [illegible] ing this problem we shall make further [illegible] studies and shall report our findings [illegible] future

It is considered likely that the dist[illegible] the hypothalamic function in other [illegible] change in hypothalamic excitation [illegible] more closely related to the disturba[illegible] autonomic nervous system the distu[illegible] the endocrine system and psychiatric [illegible]

The hypothalamic excitation is as[illegible] modify the level of the vestibular [illegible] and influence the incidence and deve[illegible] of vertigo and/or dizziness

ACKNOWLEDGEMENT

Gratitude is expressed to Assistant Profess[illegible] Matano for helpful suggestions during th[illegible]

ZUSAMMENFASSUNG

Die elektrische Reizung des Hypothalamus be[illegible] übt einige Einflüsse auf die Tätigkeit der [illegible]

ien je nach der Reizungsordnung aus Vestibulare ien wurden durch die Reizung im anterioren n Hypothalamus Uberwiegend erregt wahrend die g im mittleren medialen Hypothalamus haupt d e Abnahme von Entladungsfrequenzen in den laren Neuronen ausloste Die Moglichkeiten der Arten von hypothalamischer Einflusse auf die ves Erregbarke t werden d skutiert

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T Matsunaga M D
Dept of Otolaryngology
Kansai Rosai Hosp tal
Inabaso 3 1-69
Amagasaki City
Hyogo Pref
Japan

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THE EFFECT OF GRAVITY ON POSITIONAL ALCOHOL NYSTAGMUS PHASE II IN MAN

L M Odkvist

From the Department of Otolaryngology Regional and University Hospital Linkoping Sweden

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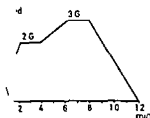
Abstract On request from the Swedish state authorities for health and civil air transport (Socialstyrelsen and Luftfartsstyrelsen) experiments were performed in order to establish the duration of the vestibular disturbances after alcohol intake related to rules for aviation safety It is known that the threshold for provocation of positional alcohol nystagmus (PAN) is lowered under the influence of gravity forces above normal It has been suggested that PAN II could be provoked by *g* loads as late as 48 hours after a small alcohol intake Our experiments were performed in a human centrifuge with *g* values 2 and 3 Whisky (43 vol% 2 5 cc/kg b w) was given in a single dose and blood alcohol analyses were frequently taken The results show that vestibular disturbances appearing as *g* provoked PAN II do not persist for any considerable time Consequently the present limit of 24 hours sobriety before flight seems sufficient Positional nystagmus provoked by *g* load even without alcohol intake in the test persons is a new observation

As early as 1842 Fluorens described nystagmus as one of the consequences of alcohol intoxication Barany (1911) and Barany & Rothfeld (1913) pointed out that this alcohol induced nystagmus was dependent upon the position of the subject s head when examined In a series of investigations Aschan et al (1955 1956 1958 and 1961) in controlled human experiments using ENG and simultaneously monitoring the blood alcohol curve showed that after a single dose of alcohol positional alcohol nystagmus (PAN) could be recorded and quantitatively analysed For details the reader is r[illegible] the references given above It is ess[illegible] the purposes of the present investig[illegible] PAN II should persist as late as 48 h[illegible] the last alcohol intake An interval of[illegible] between the last alcohol ingestion a[illegible] activity should according to thes[illegible] ments guarantee that the vestibul[illegible] quences of alcohol intoxication are [illegible] and the pilot fit for flying duties

Bergstedt (1961) showed that *g* val[illegible] 1 36 *g* increased the intensity of PA[illegible] a few experiments even PAN II [illegible] mentioned experiments were few a[illegible] performed relatively soon after the al[illegible] take when alcohol still was prese[illegible] blood Bergstedt also showed in pa[illegible] ing positional nystagmus other than P[illegible] it often intensified when the *g* load [illegible] creased In healthy normal test [illegible] without alcohol *g* loads did not [illegible] nystagmus in Bergstedt s experiment[illegible] used a human centrifuge so arranged [illegible] gular acceleration as a source of error [illegible] excluded

Oosterweld et al (1969 1969 and 19[illegible] shown that PAN I and PAN II are abo[illegible] weightlessness as reached in parabol[illegible] They also investigated a few test pers[illegible] ing the period after PAN II increa[illegible] *g* value to 3 by flying in tight circles [illegible] speed They report nystagmus find[illegible] *g* load resembling PAN II up to 48 [illegible]

The experiments were supported by Swedish Medical Council grant number B71-40X 3305 01G (1368/70 Aschan)



Relationship between angular speed resultant ...nd time during every experiment

...ng the alcohol intake At present the ... during which alcohol drinking is forbid... r pilots is 24 hours before flight, but the ... of Oosterweld et al would double this ... Hence a thorough investigation was ...ted by Luftfartsstyrelsen and Social ...en

MATERIAL AND METHODS

...een persons have been used in com...sts 12 men aged 20–36 years (mean age ... ody weight 54–91 kg (mean 74) 5 wom...d 18–26 years (mean 21) body weight ... (mean 60) The test persons were vol...s from the Police School the Medical School and the University of Stockholm The centrifuge was the one at the Aviation and Naval Medicine Institution Karolinska Institutet Stockholm used by Bergstedt (1961) to whom the reader is referred for details The test person had the force resultant from gravity and centrifugal force in his binaural line The g values used were 2 and 3 g The angular acceleration never exceeded 0 9°/sec² so as not to induce rotatory nystagmus Supporting cushions were placed at the forehead and occiput to avoid coriolis forces Usual ENG technique described by Aschan et al (1956) was used The test leader was in constant contact with the test person by a two-way intercom and by a TV camera watching the test person s head and neck Before every new registration blood samples were taken to disclose any (forbidden) alcohol consumption

The test procedure was as follows

Before alcohol intoxication

1 An interview was made in order to exclude test persons with illnessess especially related to hearing and balance medication or suspected alcohol abuse No alcohol intake

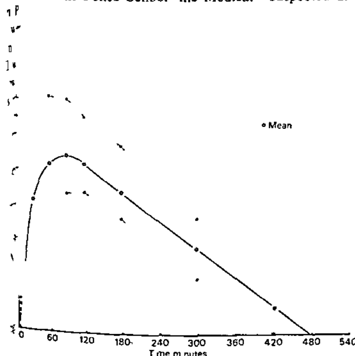


Fig 2 Blood alcohol curves The unbroken line shows the mean value for all the different test persons alcohol curves The maximum and minimum alcohol curves are also drawn

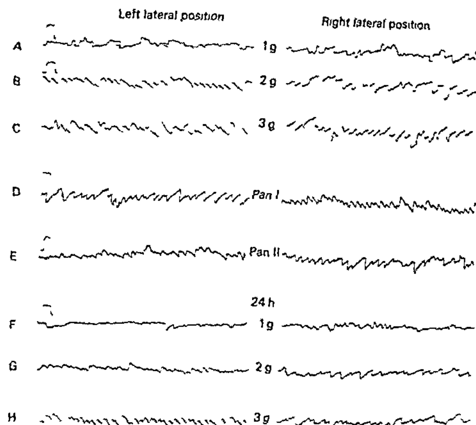


Fig. 3. Sequence of electro nystagmogram test person group 2. Right beating nystagmus in left lateral position and left beating nystagmus in right lateral position at 2 and 3 *g* (B and C) in the sober test person and no positional nystagmus at 1 *g* (A). D shows PAN I and E PAN II. After *24 hours the nystagmus at 2 and 3 g* (F and G) [...] with B and C. The calibration for 10° eye m[...] *each registration makes this quantitative* [...] ble. The blood alcohol concentration was a[...] and at E 0.67 0/00.

was permitted for 48 hours preceding the experiment.

2. Electronystagmography (ENG) was performed with the test person lying back and in right and left lateral positions with closed eyes.

3. ENG-registrations without alcohol were made in the centrifuge under *g* values 2 and 3 with the test person in right and in left lateral position. The running schedule is presented in Fig. 1.

Alcohol intoxication and later

4. 2.5 cc whisky per kg body weight was ingested in 10 minutes. Blood alcohol test was taken before the alcohol administration and frequently thereafter (see Fig. 2).

5. Registration by ENG of PAN I and PAN II was performed.

6. Centrifuge experiments with *g* [...] and 3 were performed several times [...] II could no longer be evoked. Right [...] lateral positions were used accord[...] schedule in Fig. 1.

Blood alcohol analyses were tak[...] each test person frequently enough [...] struct a blood alcohol curve for each [...] ject (see Fig. 2). The analyses were pe[...] by gas chromatography at the insti[...] clinical chemistry, Regional and L[...] Hospital of Linkoping. This method [...] and accurate results with very small [...]

RESULTS

The unintoxicated test persons were [...] gated in right and left lateral positi[...] and 3 *g*. Twenty different test persons [...]

The test persons reacted in four dif ways

up 1 In six tests the subjects did not any nystagmus at any of the g values 1 2

up 2 Nine test persons were free from mus at 1 g but had nystagmus findings at g This nystagmus was in all the eleven ments right beating nystagmus in left position and left beating nystagmus in ateral position which could have been erpreted as PAN II if pre alcohol tests ot been performed

up 3 Even at 1 g 2 test persons showed onal nystagmus similar to that described for group 2 This nystagmus appeared 2 and 3 g

e test persons had to be excluded be at 1 g they showed a spontaneous *mus uninfluenced by position* They ested unintoxicated at 2 and 3 g but as f them had a syncope during g loading were all excluded

each test person the time was noted PAN under g load was last seen and the test showed absence of PAN For 2 and 3 absence of PAN is replaced nystagmus comparable to the pre ation findings The mean duration of riod from alcohol ingestion during which II could be re evoked by g loading was oup 1 18 hours and for group 2 19 hours test finding of PAN under g load was 24 in one single test person but he belonged oup 3 and the pathological positional mus in the *unintoxicated* state is a of error

DISCUSSION

N II is almost exclusively seen in man 196? Ödkvist & Oosterweld in prepara he experiments had to be performed on test subjects Furthermore the experi were performed in order to solve prob concerning aviation safety In order to be obtain good *recordings without unnec*cessary disturbances and risks *for the test* persons we used a centrifuge to produce the g values instead of the other expensive possi bility—that is an airplane The choice of centrifuge experiments instead of airplane ex periments appeared to be successful As seen in Fig 1 the g loads could be so prolonged as to eliminate rotatory provoked nystagmus as a source of error Slow *accelerations from 1 to 2* g and from 2 to 3 g were chosen in order to avoid rotatory induced nystagmus

The reaction pattern in group 2 has never been described before in a sober test person showing no positional nystagmus at 1 g but obtaining positional nystagmus at 2 and 3 g

The possibility that the findings were differ *ent if the test person had not only the head but* also the body in lateral position was investi gated This was done on the suspicion that with the head in lateral position but the body not totally turned nystagmus of a cervical origin might appear No difference was seen however between these two variations of pos ition in the centrifuge The clean conditions in centrifuge experiments compared with air plane experiments do not even in this con nection produce as great risks for sources of error

The greatest sources of error are excluded by using in every experiment the same centrifuge the same position and the same schedules for the alcohol intake and the g loads In order to avoid habituation the test procedure for every volunteer could not be repeated too often It should be observed that *the nystagmus findings in groups 2 and 3 under* g load were always of the right beating type in left lateral position and left beating nystagmus in right lateral position These nystagmus di rections are the same as is shown by positional alcohol nystagmus phase II (PAN II) without the 0-experiments these test persons some hours after the alcohol intake could have been mistaken as still having PAN II during a load of higher g values The doses of alcohol were systematically related bo body weight in order *to yield the same level of intoxication in the*

different test persons The dose was chosen to give a maximum blood alcohol level punishable for car drivers according to Swedish law The dose chosen was based on some thousand earlier human controlled alcohol experiments (Aschan et al , 1956, 1957, 1958, 1961 and 1964) The test persons were instructed not to touch alcoholic beverages for 48 hours before the procedure but the test showing no blood alcohol before the experiment was the only way to exclude earlier PAN II as a source of error Fig 2 shows that the amount of alcohol administered resulted in a blood alcohol concentration that according to Swedish law for car drivers is criminal The figure also shows that although the given amount of alcohol per kg bodyweight was the same, and ingested into an empty stomach, the spread in blood alcohol concentration among the test persons was considerable

The conclusion of the experiments is that PAN II can be re-evoked by higher *g* values No proof has been found, however, that PAN II can be evoked after a longer period of time—24 hours between alcohol intake and flight seems to be enough This is valid for an alcohol intake not exceeding 2 5 cc alcohol per kg body weight i e 20 cl whisky for a man of 80 kg The finding that 9 sober test persons out of 17 not having any positional nystagmus when *g* loaded showed findings identical to PAN II, was a new and surprising observation This might be a source of error in similar experiments if not considered

ACKNOWLEDGEMENTS

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ZUSAMMENFASSUNG

Experimente zur *Dauer vestibularer Storungen* nach Alcoholkonsum wurden durchgeführt um brauchbare Daten für die Flugsicherheit zu gewinnen Es ist bekannt dass die Provokationsschwelle des Alkohollagenystagmus bei *g*-Belastungen gesenkt wird Man hat F[illegible] publiziert die Alkohollagenystagmus [illegible] erhöhter Schwerkraft wahrend 48 Stunden [illegible] Alkoholkonsum gezeigt haben Wir gaben Whisky (43 vol% 2 5 cc/kg) und bestimm[illegible] koholspiegel haufig Unsere Experimente Zentrifuge (2 und 3 *g*) *zeigen g provozier*[illegible] lagenystagmus dauert nicht länger als die [illegible] geforderte Sicherheitszeit für Piloten von [illegible] bedeutungsvoller neuer Befund war dass die [illegible] Personen ohne Alkoholeinfluss unter [illegible] Lagenystagmus zeigten

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L M Odkvist M D
Dept of Otolaryngology
Regional and University Hospital
S 581 85 Linkoping
Sweden

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s of the organ of Corti were dissected the temporal bones, stained by a modifi n (*vide infra*) of the silver pyridine od of Holmes (McManus Mowry, 1960) studied as whole mount or surface prepara with the light microscope The experi al material was compared with similarly ed preparations from normal animals ie experimental lesions were made in the al aspect of the brain stem by the method ibed in our previous study (Wright & ton 1973) Successful lesions were rmed in 14 animals The post operative val times ranged from two days to four s however most animals were allowed rvive from one to three weeks The exact val times are given in the following table

perative al time (days)	Number of animals
[illegible]	
[illegible]	1
[illegible]	1
[illegible]	3
[illegible]	4
[illegible]	1
[illegible]	3
[illegible]	1
[illegible]	

each case the extent of the lesion was fully determined by a study of brain stem ons prepared according to the method of & Eichman (1959) Fig 1 illustrates ramatically the positions of the lesions In t animals the lesion extended inward from surface of the brain stem just lateral to the us limitans to an area just inside the spinal t of the fifth cranial nerve (position indi d by the solid wedge) In four cases the on had a more medial position and included descending root of the facial nerve as indi d by the broken wedge In all animals the on extended approximately one millimeter ral and caudal to the level of the genu of facial nerve

he cochleas on the side contralateral to the on were processed in parallel with those n the operated side Additional control

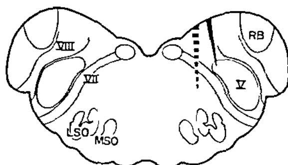


Fig 1 Diagrammatic cross section of guinea pig brain stem at the level of the facial genua The solid and broken wedges upper right indicate the positions of lesions discussed in the text *VIII* vestibulo-cochlear nerve *VII* facial nerve *V* nucleus and spinal tract of trigeminal nerve *LSO* lateral superior olivary nucleus *MSO* medial superior olivary nucleus *RB* restiform body

material was provided by preparations obtained from a large number of normal guinea pigs (approximately 25 animals) stained by several different silver methods

Many variations of standard silver stains for unmyelinated nerve fibers were tried in the course of this work The procedure which we found most consistently useful is as follows

1 Fix inner ear tissues by perilymphatic perfusion with 10% neutral formalin (stored over calcium carbonate) Allow the temporal bones to remain in formalin for at least 4 weeks at refrigerator temperature (2–6°C)

2 Remove temporal bones from formalin and perfuse with 5% trichloroacetic acid and leave in this solution 1–2 hours at refrigerator temperature

3 Wash well in distilled water and dissect pieces of organ of Corti from the cochlea

4 Place pieces in a staining solution made from 1 ml of 1% silver nitrate 0 5 ml of 10% pyridine and 48 5 ml of distilled water (pH of final solution 6 5 to 7 0) for 5 days at 37°C

5 Transfer without washing to a reducing solution containing 10% sodium sulfite and 1% hydroquinone for 5 minutes at room temperature

6 Wash well in 10% sodium chloride

7 Transfer to 0 5% gold chloride for 20 minutes at room temperature

8 Wash briefly in 10% sodium chloride

9 Place in fresh reducing solution (step 5) for 5–10 minutes

10 Wash with 10% sodium chloride and mount in glycerol

All the above solutions are prepared with distilled water

With this method both afferent and efferent neural elements in the inner spiral bundle region were stained against a suitably light background in whole mount preparations In this material it was possible to survey large portions of the organ of Corti permitting small fascicles of fibers to be traced over long distances It is recognized however that this technique like other silver methods presents certain disadvantages In a given preparation not all the nerve fibers present become stained making quantitative studies unreliable Also small fascicles composed of several fibers often stain as a single unit making it difficult to determine the precise course of individual fibers On the other hand when the very dense innervation in the area of the inner hair cells is to be studied it is actually preferable that all fibers do not stain because of the simplification that results Finally the emphasis in the present investigation is on clarification of the overall wiring plan in the inner spiral bundle region rather than on a determination of the exact lengths of individual fibers For this purpose the above method was found to be useful

RESULTS

Previous workers have shown that in the region just distal to the habenula perforata and beneath the inner hair cells of the organ of Corti several different groups of nerve fibers are found closely packed together Bundles of afferent fibers emerge from the habenula and distribute themselves in a predominantly radial fashion to the inner hair cells Some afferent fibers course directly outward between the inner pillar cells to innervate the outer hair cells Other nerve fibers which experimental studies have indicated are of an ef[illegible] ture either enter the inner spiral bun[illegible] to course for variable distances below [illegible] ner hair cells or run straight out [illegible] habenula into the tunnel of Corti and [illegible] the outer hair cells as upper tunnel [illegible]

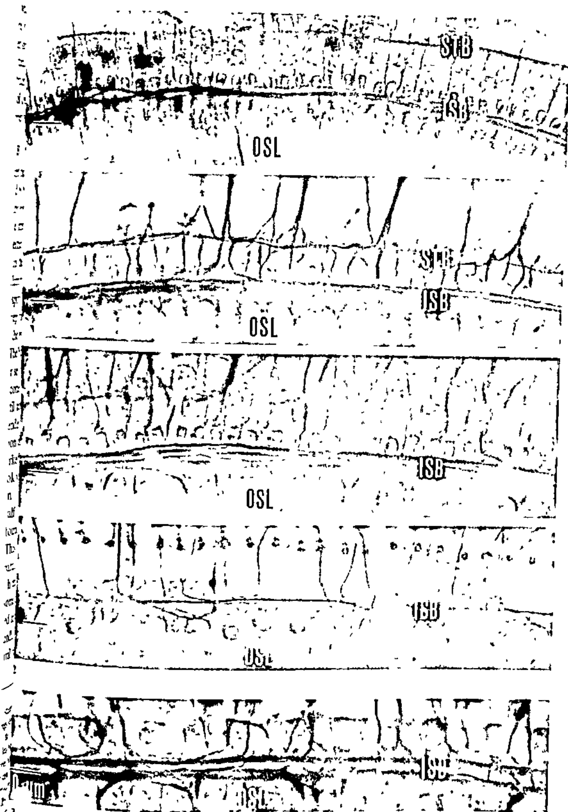
General morphology of ISB

In normal animals the light microsc[illegible] of this region is dominated by the in[illegible] bundle This bundle of unmyelinated fi[illegible] cated directly beneath the inner ha[illegible] not to be confused with either the [illegible] lionic spiral bundle within the [illegible] with the myelinated intralaminar sp[illegible] prominent in the osseous spiral lamina and some other forms Preparations [illegible] the ISB in various turns of the guinea p[illegible] lea are pictured in Fig 2 The bundle is [illegible] in the upper basal or lower second turn [illegible] guinea pig cochlea and becomes sl[illegible] ner in the upper turns until near th[illegible] narrows down to a few slender fascicle[illegible] ward the lower basal portion of the [illegible] Corti the ISB becomes markedly th[illegible] in the region of the basal hook very few [illegible] long spiral fibers can be seen

Especially in the basal half of the [illegible] Corti the ISB is frequently found to be [illegible] into several sub bundles This d[illegible] often takes the form of a separation [illegible] three discrete bundles which may re[illegible] after a short distance or in some cases separate for several hundred microns uniting again into a single bundle An [illegible] of such a divided inner spiral bun[illegible]

Fig 2 Photomicrographs of [illegible] [illegible]

ous spiral lamina STB [illegible] ral tunnel bundle [illegible] lower left represents 10 μm in each case
Fig 3 Photomicrograph of a s[illegible] [illegible] from a normal guinea pig [illegible] chlea [illegible] inner spiral bundle (ISB) in the basal turn OSL [illegible] spiral lamina


STB
ISB
OSL
STB
ISB
OSL
ISB
OSL
ISB
OSL
ISB
OSL

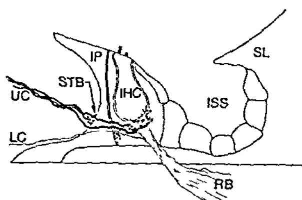


Fig 4 D agrammatic cross section drawn from a silver stained specimen showing the inner spiral bundle beneath an inner hair cell (*IHC*) In this case the ISB is divided into three large fascicles separated by a rad al bundle of fibers (*RB*) and an inner phalangeal cell (stippled) A lower tunnel-crossing fiber (*LC*) leaves the rad al bundel to run directly into the tunnel without pass ng through any portion of the ISB Upper tunnel-crossing f bers (*UC*) originate from both the ISB and sp ral tunnel bundle (*STB*) *IP* inner p llar cell *ISS* inner spiral sulcus *SL* spiral l mbus

basal turn is shown in Fig 3 Each of the divisions contains fibers running in both directions and each gives off tunnel-crossing nerve fibers Radial sections through these areas show that the ISB divides to pass on either side of the phalangeal cells and to surround the radial fascicles of fibers approaching the inner hair cells from the habenula perforata This arrangement is diagrammed in Fig 4 In areas where such a separation is not apparent the fibers of the ISB are found more or less evenly distributed among the incoming radial fascicles and the supporting cell processes

Brain stem lesions

The brain stem lesions made to interrupt the efferent innervation to the cochlea resulted in varying degrees of degeneration in the inner spiral bundle area In the animals with longer post-operative survival times these variations in amount of degeneration were unexpected since in all 14 experimental animals the lesion site appeared to include all of the region occupied by the outgoing contra and ipsi-lateral olivo-cochlear bundles—at least according to the majority of published accounts of the brain stem course of these tracts In the animals with the more laterally placed lesions (as shown in Fig 1) the degree of loss of [illegible] fibers ranged from a slight reduction [illegible] appearance of 80% or more of the fibers normally present This variation was seen in animals allowed to survive 2–4 weeks. [illegible] the four cases in which the lesion had a [illegible] medial position and extended slightly [illegible] into the brain stem was there a total [illegible] pearance of long spiral fibers from the [illegible] region and of upper tunnel-crossing [illegible] Two of these animals had survival times [illegible] days and two survived 14 days

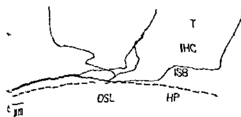
Patterns of fascicle distribution beneath the inner hair cells

Although the differing degrees of degeneration found in this series of animals were surprising the preparations obtained provided [illegible] material for the study of neural organization in the region occupied by the inner spiral bundle It was possible to distinguish several [illegible] nerve fascicles on the basis of their pattern of distribution in the region below the [illegible] cells

First there exist numerous heavily [illegible] fascicles which emerge from the habenula [illegible] run directly through the ISB without [illegible] *spiral course in the bundle Fibers of* [illegible] course to the outer hair cells as upper [illegible] crossers

Other upper tunnel-crossing fibers [illegible] short spiral course of 50 μm or less in [illegible] before making a right angle turn into the tunnel *These short spiral fascicles* [illegible] quite darkly and run either toward the [illegible] the apex of the cochlea

Two types of long spiral fascicles of [illegible] size and staining intensity could be [illegible] in the inner spiral bundle One of [illegible] *eventually turns outward to innervate* [illegible] outer hair cells The other remains with [illegible] ISB complex and ends there It was [illegible] *sible to trace slender fascicles* [illegible] only a few fibers for several hundred [illegible] without being able to discern a decrease [illegible] *their diameter* At least some of the [illegible]



Camera lucida drawing of a spiral fascicle splitting ...veral individual fibers all of which cross the tunnel ...us fascicle was found in the apical turn coursing the apex of the cochlea *OSL* osseous spiral lami... habenula perforata *ISB* inner spiral bundle ... *IHC* nuclei of inner hair cells

...nt fibers must therefore extend the entire ...n of the fascicle These small bundles of ...s were found running in both directions in ...SB and could often be followed for ...00 µm Occasionally several fibers could ...en splitting off the parent fascicle to turn ...cross the tunnel The longest spiral fasci... ...hat we were able to trace ran toward the ...m in the third and second turns and had a ...course of 1 2 mm before it was lost in the ... It could not be determined if all the fibers ...s fascicle crossed the tunnel, however, at ...int approximately 350 µm from its entry ...the ISB several fibers did split off as a ...p and run through the tunnel as upper fibers Sometimes it was possible to trace ...spiral fascicles to a point where they split into smaller units, all of which crossed to ...outer hair cells One such fascicle in the ...al turn is illustrated in Fig 5

...ue to the fact that the silver method used ...is work rarely stains the terminal portion ...e nerve fibers, it was difficult to identify ...tively spiral fascicles which ended within ...ISB region However a few fascicles were ...ed that did appear to end in this area Oth... ...could not be followed for enough to de...mine their final destination

...fferent fibers serving both the inner and ...er hair cells are present in the inner spiral ...dle region The short fibers that go more or ...directly to the inner hair cells comprise ...larger group of afferents in the guinea pig ...nerally, the afferent fibers destined for the outer hair cells cross the tunnel of Corti near its floor Some of these lower tunnel crossers split away from the fiber bundles immediately after their emergence from the habenula to run directly toward the floor of the tunnel, without passing through the ISB One such fiber is diagrammed in Fig 4 Others take a short, angular course through the inner spiral bundle of 10–30 µm (in most cases) before entering the tunnel, the longest of these fibers that was observed ran 45 µm in the ISB area Fibers of this type are shown in Fig 6, which was drawn from a preparation in which all long ISB fibers had disappeared In the few cases in which all the ISB fibers had degenerated and there was suitable staining of the remaining afferent fibers we had the clear impression that the majority of incoming fibers terminated in the inner hair cell region Only 2 to 4 fibers from each afferent fiber bundle could be found to cross the tunnel No suggestion of afferent fibers which provide collaterals to inner hair cells before crossing to the outer hair cells could be found in these preparations

A pattern of spiral fiber distribution which appeared to be confined primarily to the basal turn is illustrated in Fig 7 In this portion of the cochlea bundles of fibers are often seen entering the organ of Corti and dividing im...

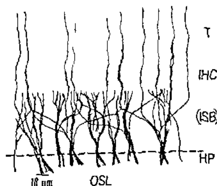


Fig 6 Camera lucida drawing of afferent nerve fibers in the area normally occupied by the inner spiral bundle (*ISB*) in a preparation in which all ISB fibers have degenerated Note that only a few fibers from each incoming radial bundle cross the tunnel space (*T*) *OSL* osseous spiral lamina *HP* habenula perforata *IHC* nuclei of inner hair cells

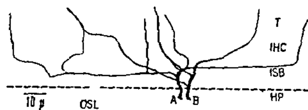


Fig 7 Camera lucida drawing illustrating the d stribution of two nerve fascicles (*A* and *B*) in the inner sp ral bundle (*ISB*) of the basal turn Together the two main divis ons

spiral lamina *HP* habenula perforata *IHC* nuclei of inner hair cells *T* tunnel of Corti

mediately into two fascicles which course in opposite directions in the inner spiral bundle After a spiral course which is usually less than 100 μm in length both fascicles turn outward to become upper tunnel crossers These espalier like formations are frequently strik ingly symmetrical with two limbs of roughly equal length In some of these formations a central limb is present which goes directly into the tunnel as shown in bundle B of Fig 8 Also the limbs of adjacent bundles may join together to form a single tunnel crossing fasci cle as seen in Fig 8

Another pattern found in the upper turns as well as in the base is illustrated in Fig 9 In this figure several bundles can be seen enter ing the organ of Corti and dividing in such a way that one portion of each bundle proceeds directly across the tunnel while the other turns into the ISB In a few cases long spiral fasci cles originating in this way could be followed

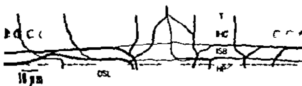


Fig 9 Camera lucida drawing illustrat ng a pattern of nerve f ber d stribution seen in both the upper and lower turns of the cochlea Several fasc cles are shown entering the inner sp ral bundle region (*ISB*) and dividing in such a way that one port on of each fascicle runs directly across the tunnel (*T*) wh le the other divis on enters the inner sp ral bundle The area shown is from the basal turn *OSL* osseous spiral lamina *HP* habenula perforata *IHC* nuclei of inner hair cells



Fig 8 Camera lucida drawing showing the dis several nerve fascicles emerging from the perforata (*HP*) and entering the inner spira in the basal turn Fascicles such as A and B and take a sp ral course in the ISB interm fasc cles wh ch run across the ISB without any spiral course in the bundle Fascicles A and B total d stribution of 80–90 μm in the ISB *OSL* spiral lamina *IHC* nuclei of inner hair cells *T* Corti

100–200 μm and then be seen to cros outer hair cells The longest such fasc could be traced to its entry into the tu 250 μm toward the base in the basal tu

DISCUSSION

ISB organization

One of the objectives of the present in tion was to study the organization o fibers in the inner spiral bundle throughout the guinea pig cochlea termine whether differences in the pa innervation exist in the different turns eral it was found that the similarities o organization from one turn to the n more striking than the differences The types of direct radial short spiral a spiral fascicles described above were a closely intermingled in all turns of the o Corti

The overall size of the ISB is fairly c through the middle turns although m preparations a slight increase in d am usually evident in the upper basal and second turns The larger size of the b this region appears to be due to a abundance of elements that give rise to tunnel-crossing fibers This is in accor the well-established observation that t

of efferent innervation to the outer hair is greatest in the lower portion of the [illegible]lea (Fernandez 1951, Schuknecht et al, Engstrom et al, 1966, Spoendlin, 1966, [illegible]h & Sjostrand, 1961) At both the apex [illegible]extreme base of the organ of Corti the [illegible]er of fibers in the ISB is markedly re[illegible]d This reduction is most pronounced in [illegible]basal hook where only a few, widely [illegible]d fascicles can be seen These findings [illegible]consistent with previous studies made [illegible]everal different species using acetyl[illegible]nesterase staining where little positive [illegible]ion was found in the ISB at the ex[illegible] apical and basal ends of the cochlea [illegible]iknecht et al, 1959, Hilding & Wersall, [illegible] Ishii & Balogh, 1968, Firbas & Wel[illegible]ik 1970)

[illegible] preparations stained by the ZIO method [illegible]trom et al (1966) have noted the occa[illegible]l division of the ISB into several discrete [illegible]les especially in the basal portion of the [illegible]a pig organ of Corti In our silver stained [illegible]nal the bundle was often seen to split into [illegible]or three large fascicles which might re[illegible]ine almost immediately or, as was some[illegible] the case, remain separate for several [illegible]red microns These splits appeared to oc[illegible] relation to incoming radial bundles or to [illegible]ngeal cell processes as illustrated in Fig [illegible]ing the electron microscope, Smith (1961) [illegible]ved bundles of ISB fibers dividing [illegible]d phalangeal cells We found that the ISB [illegible]t be divided into separate fascicles in any [illegible] but this did occur most frequently in the [illegible] half of the cochlea

[illegible]e form of spiral fiber distribution il[illegible]ated in Figs 7 and 8 was seen clearly only [illegible]e basal turn In this region, bundles of [illegible]s entered the organ of Corti and im[illegible]iately bifurcated into fascicles which ran [illegible]posite directions in the ISB After a vari[illegible] spiral course of up to about 100 μm these [illegible]cles crossed through the middle of the [illegible]el space to the outer hair cells The inner [illegible]l bundle in the basal turn often appeared [illegible]e composed of many of these units overlapping one another and intermingled with very long spiral fascicles

In addition to those in the base, it was clear that many ISB fascicles in the upper turns also left the bundle to supply the outer hair cells —often after an extended spiral course under the inner hair cells It is, unfortunately not possible with the present method to make a reliable estimate of the proportion of ISB fibers which eventually cross the tunnel However, it was obvious that this proportion is quite sizable the fibers of the inner spiral bundle certainly do not terminate exclusively in the inner hair cell region This point was also made by Fernandez (1951) in his now classical description of cochlear innervation in which he described fibers that run for 200 μm or more in the ISB

Placement of lesions

In view of the position of the brain stem lesions made in our experimental animals we were surprised to find large variations in the amount of degeneration that occurred in the inner spiral bundle region It seems unlikely that this was due to insufficient post-operative survival times since we found total degeneration of spiral tunnel bundle fibers (verified by electron microscopy) with survival times as short as 5 days (Wright & Preston 1975) Different degrees of fiber loss in the ISB even after 3 to 4 weeks were found in several animals However in the cases in which the lesions were more medially placed (as shown in Fig 1) there was total disappearance of long spiral fibers in the ISB after survival times of 1–2 weeks

Thus the variability in degeneration was associated with the more lateral lesions which extended just into the dorsal aspect of the spinal tract of the trigeminal nerve The most plausible explanation for these findings appears to be that some of the exiting efferent fibers cross in a more ventral position through the nucleus and spinal tract of the fifth nerve rather than over the dorsal aspect of these structures Such fibers would be spared by

lesions that failed to penetrate deeply into the spinal tract of V. These fibers, however, would have been included in the more medial lesions which extended somewhat deeper into the brain stem

Ross (1969, 1973) has described acetylcholinesterase-positive fibers crossing through the spinal tract of V, and other authors such as Osen & Roth (1969) and Rossi (1968) have included fibers in this position in their diagrams of the brain stem course of the efferent bundles In Rossi's diagram it is specifically the ipsi-lateral bundle that is shown to cross in the more ventral position on its way to join the eighth nerve

If this explanation for the results of the present study is correct we can then conclude that all long spiral fibers in the ISB of the guinea pig are efferent This conclusion is in agreement with the findings of Spoendlin (1966) in the cat and of Smith & Rasmussen (1963) in the chinchilla It should be noted, however, that the same results would be expected on the basis of Ross' concept of the parasympathetic innervation of the cochlea and that neither of the two types of lesions made in the present study is useful in deciding the question of the true origin and nature of efferent fibers supplying the inner ear

Afferent fibers

The limited observations we were able to make of afferent nerve fiber distribution in the absence of any inner spiral bundle fibers lend support to the conclusions of Spoendlin (1972) regarding the proportion of afferent fibers that end on the inner hair cells His results indicate that the vast majority (about 95%) of all afferent fibers to the organ of Corti in the cat terminate on the inner hair cells In our experimental preparations it appeared that only three or four fibers from each incoming bundle of afferent fibers crossed the tunnel to the outer hair cells This observation matches almost exactly the electron microscopic findings of Iurato & Smith (1970) in the chinchilla cochlea

In preparations in which all effer[…] had been eliminated we were unab[…] giant fibers innervating the inner ha[…] the type Spoendlin (1972) has descr[…] cat Also no suggestion of afferent f[…] might have endings on both inner a[…] hair cells could be found in our ma[…] were thus unable to find anatomica[…] for the interesting suggestion recen[…] by Nieder & Nieder (1970) regard[…] istence of fibers that might be excite[…] the inner and outer hair cells

Finally, it is of interest to note […] types of afferent tunnel crossers ex[…] tion to the inner spiral bundle Those […] that take a short spiral course before […] actually enter the ISB and could the[…] contacted by efferent fibers of the […] However, many afferent crossers s[…] from the radial bundles immediately a[…] come through the habenula and run […] toward the tunnel without passing thr[…] portion of the ISB Such fibers can b[…] identified in radial sections of the […] Corti such as illustrated in Fig 4 […] therefore little possibility of synaptic […] tion between these fibers and those o[…] ner spiral bundle

ACKNOWLEDGEMENT

The author wishes to thank Prof Joseph E H[…] for his thoughtful suggestions and encourageme[…] out the course of this study

The assistance of Verne T Maulbetsch […] Combs and James W Bruce in preparation of […] is also gratefully acknowledged

ZUSAMMENFASSUNG

Die Organisation der Nervenfasern in der Lag[…] inneren Haarzellen der Meerschweinchenschn[…] an Hand von silbergefarbten Flachenprapar[…] mikroskopisch untersucht Es wurde gefunden […] innere Spiralbundel (ISB) in allen Windu[…] Cortischen Organs sowohl kurze als auch lan[…] fasern enthält die entweder zur Basis oder […]

ISB Fasern kreuzen den Cortischen Tunnel zu den ?en Haarzellen Operative Unterbrechung der ef en Nervenversorgung im Hirnstamm fuhrte zur De ition aller ISB-Fasern Nach der Eliminierung der ?nten Fasern konnte beobachtet werden dass die ?ahl der afferenten Fasern auf den inneren Haar end t

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Ch G Wright Ph D
Dept of Otolaryngology
Kresge Hearing Research Institute
University of Michigan Medical School
Ann Arbor Mich 48104 USA

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NOISE AND THE RNA SYNTHESIS OF THE COCHLEA

Autoradiographic Studies

H Kraus, W. Richrath, L Dullweber, D Gerbig,
R Prieshof and J Tillmann

From the Department of Otolaryngology University of Munster Munster BRD

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Abstract In cochlear basal turns of 22 guinea pigs with autoradiography, grain density was measured after injection of ^{3}H-cytidine Following high initial nuclear labelling a low incorporation in cytoplasm was observed even after 24 hours Synthesis of ribosomal RNA in the organ of Corti seems to be slow Low grain densities in the whole cochlea presuppose a blood-lymph barrier to cytidine After white noise (85 dB SPL 12 hours) were seen a more rapid incorporation into nuclei and after 24 hours an increased labelling of cytoplasm of ganglion cells fibrocytes and inner hair cells less of outer hair cells This might be induced by increased blood flow, concentration of the precursor in the lymphs cell permeability or alterations of the cellular nucleotide pool But a real stimulation of cochlear RNA synthesis seems to be the most likely On comparing different turns a peak in grain density was found over nuclei of the organ of Corti after 1 hour in the 2nd turn After 24 hours cytoplasmic labelling showed a decrease only in outer hair cells towards apical turns

After the first report about the histopathology of noise damage by Habermann (1890), Wittmaack in 1907 assumed a "disturbance in the metabolism of the cell" Countless studies of enzyme histochemistry could not disclose the nature of this disturbance Thus Wittmaack's random formula came to be cited almost literally up to the present day

Many histochemical observations (Kluyskens, 1954, Beck & Michler 1960 Vinnikov & Titova, 1963, Nakamura, 1967, Ya 1967) and measurements of nucle (Neubert & Wüstenfeld, 1955, Wuse Halbfas, 1965, Anicin, 1968) assume th metabolism of the membranous cochl promptly to loud noise After intense s tion, cytophotometric studies reveale crease in cytoplasmic RNA concen spiral ganglion cells (Hamberger & 1945, Hammer, 1958, Uno, 1958 O 1960, Kon, 1964, Pakkenberg & Th 1964, cf Kraus & Gerlach, 1970) H both concentration and base compos mained biochemically unchanged (Ha al , 1965) In autoradiography with RN cursors only occasional nuclei in Corti have so far been labelled (Koburg Watanuki et al , 1968, Ruben, 1969 c Bejdl & Krejči, 1953) Following applica streptomycin or kanamycin incorpora creased (Watanuki et al 1968, Rub Kraus, 1973) Our knowledge of RN tabolism of the cochlea is therefore compared with that of other organs RNAs are key molecules in the reg of cell metabolism we have used toradiography with ^{3}H-cytidine in ord study the kinetics of RNA synthesis i cochlea for 24 hours during rest and noise exposure

This study was supported by grants from the Deutsche Forschungsgemeinschaft

Abbreviations RNA = ribonucleic acid gd = grain density



Fig 1 Autoradiogram of the organ of Corti from a guinea pig 1 hour after ^{3}H-cytidine following noise of 85 dB Only cell nuclei are labelled Unstained phase contrast objective ×40 magnification ×1 040

METHODS

camera silens 22 juvenile guinea pigs ing 110–150 g were offered only water ad nd exposed to white noise of 35 dB SPL decapitation 1, 2, 3, 4, 8 and 24 hours i p injection of 5-^{3}H-cytidine (10 0 kg specific activity 27 3 Ci/mmol, rsham) Preceding injection one half of nimals were exposed to noise of 35 dB 2 hours (controls=*C*) the other half to of 85 dB (noise=*N*) A random noise rator, type 1402, Bruel & Kjaer was used, width 20 Hz – 20 kHz, linearity up to 20 was checked with an audiofrequency rograph, type FNA, Rohde & Schwarz fixation of the cochlea with glu dehyde for 2 hours, rinsing in buffer mbedding in Epon under standard condi , 2–3 μm sections cut with an ultra otome without previous decalcification, covered with Agfa Gevaert emul NUC 7 15, exposed 9 months at 4°C, de ped fixed and embedded in oil of identical active index (Kraus et al , 1973) without ing

rain density (gd) over nuclei was measured an incident light microscope photometer right field (Dormer et al , 1966, condensor with fixed aperture of approx 0 3, condensor aperture diameter 6 7 μm, measuring aperture diameter 4 8 μm, 539 nm) Average reflexion values were checked against a standard and converted into number of grains/16 μm² by use of a calibration curve Due to non linearity in the lower measuring range, the scale for nuclei is slightly distorted in Figs 2–4 Because of weak labelling we had to count through an eye-piece over the cytoplasm (phase contrast, oil, objective ×100 mesh ocular ×20 of the field area 16 μm²) Measurements of cyto plasm of cells of tympanic lamina had to be omitted due to their irregular form In stria epithelium dark cells were omitted In ganglion cells only nuclei without nucleolar labelling were measured For remaining cell types, measured values represent the mean for both nucleoplasm and nucleoli Given mean values are derived from 3 petrous bones of 2 animals with at least 80–300 nuclei or 100–300 fields of 16 μm² in cytoplasm

Statistical evaluation was performed for nuclei with U-test, for cytoplasm, after checking for Poisson distribution, with chi² test employing c_i transformation of mean values Meaning in Figs 2–4 0=P>0 05, +=P−0 01−0 05,++−P<0 01

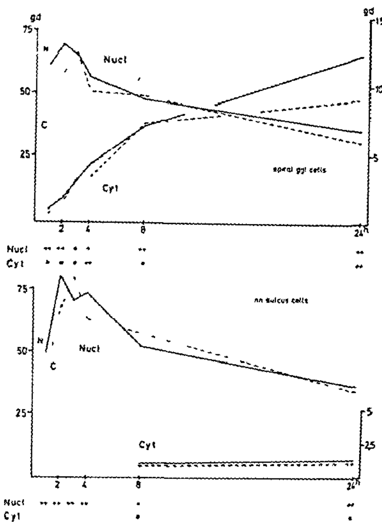


Fig 2 Time course of grain de... *scissa* Hours after i p injec... tidine *Ordinate* Grains/16 µm² at left, ×5 higher for cytopla... right Controls = *C* (12 hours bef... to 24 hours after injection 35 d... = *N* (12 hours before injection ... 24 hours thereafter 35 dB)

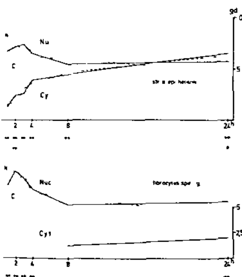
DISCUSSION OF ERRORS

A non-specific binding of the precursor as a result of fixation seems to be insignificant and would have resulted already in labelling of cytoplasm after 1 hour Control of specifity of gd with RNAse is not possible in Epon sections (cf. Kraus & Gerlach, 1970) The influence of β-self-absorption on the relation gd nucleus to cytoplasm is probably weak, since a homogeneous permeation of cells can be expected in Epon sections (refractive index 1 54, Kraus, 1970, Kraus et al., 1973) Despite extremely long exposure, the labelling of background was only 0 34 grains/16 μm^2 for controls and 0 35/16 μm^2 after noise with a low standard deviation For this reason no corrections for background activity have been made.

Differentiation between nucleus an... plasm in stria epithelium and betwee... plasm and intercellular space in fibro... spiral ligament was difficult to make... ured values are therefore probably too... the first case, and too low in the ... Nuclei of cells of tympanic lamina w... only ones which were smaller than the ... ing blend. They are therefore hardly co... ble

RESULTS

In autoradiograms after 1 hour, n... particularly of ganglion cells, are more... sively labelled than is nucleoplasm Th... plasm shows no incorporation (Fig 1) ...



3 For caption cf Fig 2

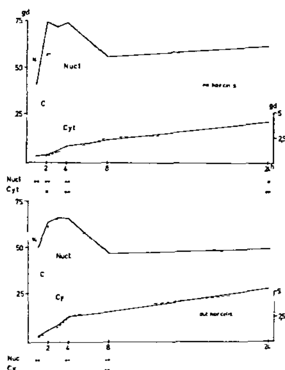


Fig 4 For caption cf Fig 2

Schwann cells, all nerve fibres remain un
lled for 24 hours

s the graphs show, we find in all cell types
he cochlear basal turn a rapid initial in
se of gd over nuclei, with a peak reached
r about 3 hours, while only in cells of the
panic lamina and inner hair cells is this
k reached later on (Figs 2–4) Thereafter
gd decreases slightly biphasically up to 24
rs In the cytoplasm the first grains appear
r 2–4 hours Their density increases up to
ours Following noise, with the exception
tria epithelium and inner hair cells, the
ear peak is reached as early as after 2
rs and cytoplasmic labelling is enhanced
t the time of maximal nuclear labelling,
e are neither significant differences be
n gd of different cells (Tab I, cols 3–4),
between noise and control (col 6) In
plasm the highest incorporation is
rved after 24 hours in ganglion cells, the
est in sulcus epithelium (cols 7–8) Follow
noise the highest percentage increase in
plasm after 24 hours is seen in ganglion
inner hair cells and fibrocytes, less in
r hair cells and stria (col 9) The portion
aximal nuclear labelling which appears in
cytoplasm after 24 hours, amounts to
14–18% for ganglion cells and stria Values for cells of Corti s organ are extremely low (cols 10–11)

As regards nuclear incorporation a significant difference is registered for different turns (Fig 5) With the exception of outer hair cells, the whole organ of Corti shows a faster nuclear labelling in the 2nd turn This maximum is raised after noise After 24 hours cytoplasmic gd shows a significant decrease from basal to apical turns only in outer hair cells, while other cells do not indicate a definite gradient

DISCUSSION

Precursors of ribosomal RNA, the bulk of cytoplasmic RNA, are synthezised in the nucleolus those of messenger- and transfer RNA in the nucleoplasm They are later transferred into cytoplasm for protein synthesis (cf biochemical reviews) The kinetics of cochlear labelling with ^{3}H-cytidine is in agreement The

Table I *Relation of grain density between different cell types of controls (C) and after* [illegible]

[illegible]

cytidine (col 5) and at time of maximal labelling (col 6)
Cols 7–8 Relation of gd in cytoplasm, 24 hours after cytidine Ganglion cells = 100%
Col 9 Percentage [illegible]
cytidi[illegible]
Cols [illegible]
labelli[illegible]

	Nucleus						Cytoplasm			Cyto. to Nucl. [illegible]
	Relation gd, 1h (%)		Relation gd, Maximum (%)		Increase *N* to *C* (%)		Relation gd 24h (%)		Increase *N* to *C* (%)	Rel. Cyto. 24h to Nucl. (%)
	C (1)	*N* (2)	*C* (3)	*N* (4)	1h (5)	At maximum (6)	*C* (7)	*N* (8)	1st–4th turn (9)	C (10)
Ganglion cells	100	100	100	100	+79	+ 5	100	100	*+34*	[illegible]
Inner hair cells	72	67	97	107	+67	+16	35	33	*+20*	5
Outer hair cells	102	82	100	96	+47	0	55	43	*+10*	[illegible]
Deiters cells	117	91	104	105	+40	− 6	24	20	*+ 2*	[illegible]
Claudius cells	98	74	104	98	+36	− 5	18	15	*+18*	[illegible]
Inner sulcus epithelium	113	81	121	116	+25	0	12	11	*+ 8*	1
Cells of tympanic lamina	61[a]	35[a]	44[a]	42[a]	+ 5	0	–	–	–	–
Stria epithelium	80[a]	60[a]	70[a]	57[a]	+30	−15	69	53	*+12*	[illegible]
Fibrozytes of spiral lig	92[a]	63[a]	68[a]	67[a]	+29	+ 2	18	17	*+30*	[illegible]

[a] Cf discussion of errors

same course of incorporation has been observed in a great number of cells in autoradiographic studies (for review Schultze, 1969) Thus RNA synthesis of the cochlea seems to be similar to other organs

Here and previously (Koburg, 1961, Ruben, 1969, Richrath & Kraus, 1973) a very low labelling of the cochlea was seen in comparison with other organs High doses of ³H-cytidine and a very long exposure were necessary This might be due not only to a blood–brain barrier (Schultze et al, 1972), but also to a blood–lymph barrier for cytidine Unlike other organs, a very low and late labelling in cytoplasm has been seen in the cochlea Similar findings were reported in biochemical and autoradiographic studies only of the retina (Koenig, 1971, Bondy, 1972) Thus a slow synthesis of ribosomes may be typical for sensory receptors *In addi*[illegible] absence of labelling of nerve *fibres* [illegible] slow axo plasmatic flow of RNA in [illegible] lea Another cause for low labelling [illegible] plasm may, however, be a relativ[illegible] synthesizing activity in nucleopla[illegible] fortunately, grain counts do not allow [illegible] quantitative conclusions Exact [illegible] blood flow, kinetics of specific act[illegible] cytidine in lymphs, of cellular nucl[illegible] pools, and of the limiting transport of [illegible] cursors through cell membranes are not [illegible] able for the inner ear Re utilization of [illegible] precursors and the existence of a se[illegible] pool for nucleus and cytoplasm are still [illegible] of argument However following the [illegible] plication of noise hair cells showed a[illegible] crease, and only after prolonged stim[illegible] decrease in RNA concentration in the [illegible]

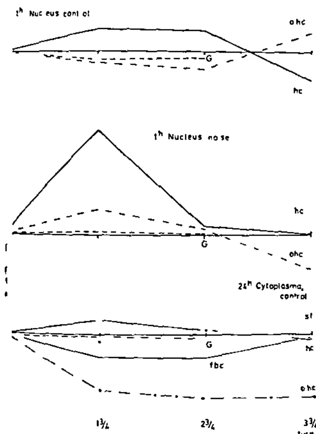


Fig 5 Percentage increase or decrease of grain density in various turns compared with basal turn (=100%) *o hc i hc* =hair cells *G* ganglion cells *st*=stria epithelium *fbc* =fibrocytes of spiral ligament

photometric measurements (Ohhashi, Kon, 1964, cf introduction) Since we a very low SPL, a real stimulation of *synthesis* by noise is most likely here hair cells reacted more strongly outer hair cells The absence of a correla between cytoplasmic gd and RNA con ation in both (Kraus & Doennig, 1969) be explained by ' wastage in the ribo- cycle''

e nuclear labelling of Corti's organ ed a significant peak in the 2nd turn her concentration of the precursor in the hs of the middle turns is conceivable s missing at least, in the case of re pyridine nucleotides (Matschinsky & nann, 1967) A differing hydrodynamic in middle turns has not been proven le, faster nuclear incorporation in the c⁵ must be caused by local alterations of permeability, nucleotide pool or RNA

synthesis Unlike cell nuclei, cytoplasmic gd of outer hair cells decreased towards apical turns The same was observed by Meyer zum Gottesberge et al (1965) for protein synthesis of cochlear ganglion cells However, we found gd in ganglion cells of different turns identical

The synthesis of all enzymes, including those of known transmitters (Fonnum et al, 1973) is RNA-dependent An important part of RNA synthesis in ganglion cells seems to be linked to the function of acetylcholine (Gisiger, 1971) Thus a depletion of RNA dur ing stronger stimulation may be one factor in the »disturbance of cell metabolism'', before the organ of Corti is mechanically damaged Our investigation of the kinetics of RNA synthesis in the cochlea, as well as that of the breakdown of RNAs and acetylcholine (Schubert et al, 1970 γ-aminobutyric acid? Richrath & Kraus, 1974), agrees so far wi the kinetics of noise trauma An ex

tion of RNA is more likely the cause of noise-induced permanent threshold shift (NIPTS) than of temporary threshold shift (TTS)

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ZUSAMMENFASSUNG

Autoradiographisch wurde an 22 Meerschweinchen bis zu 24 Stunden nach Gabe von ^{3}H-Cytidin bei weißem Rauschen von 35 dB SPL die Korndichte an Zellen der Basalwindung der Cochlea gemessen Es kam bis 24 Stunden nur zu einer niedrigen Inkorporation im Cytoplasma Im Cortischen Organ erscheint damit die Synthese ribosomaler RNA träge Die niedrige Markierung der gesamten Cochlea laßt eine Blut Lymph Schranke für Cytidin vermuten Nach Beschallung (85 dB 12 Stunden) fand sich prompt eine beschleunigte Inkorporation in Zellkerne mit erhohter Korndichte nach 24 Stunden im Cytoplasma von Ganglienzellen Fibrozyten und inneren Haarzellen weniger in äußeren Haarzellen Als Ursache kommen in Frage Zunahme der Durchblutung der Konzentration des Cytidins in den Lymphen der Zellpermeabilitat und Abweichungen im Nucleotid Pool der Zellen Am wahrscheinlichsten ist aber eine echte Stimulierung der RNA Synthese Beim Vergleich verschiedener Windungen fand sich in Zellkernen nach 1 Stunde eine beschleunigte Inkorporation in der 2 Windung besonders nach Beschallung Im Cytoplasma war nach 24 Stunden nur in äußeren Haarzellen ein Abfall der Markierung nach apikal nachzuweisen

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Dr med H Kraus
Dept of Otolaryngology
University of Munster
D 44 Munster
BRD

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EVOKED RESPONSES FROM INFERIOR COLLICULUS AS AN INDEX OF HEARING THRESHOLDS IN GUINEA PIGS

K Makishima, J C Saunders and J B Snow Jr

From the Department of Otorhinolaryngology and Human Communication the University of Pennsylvania School of Medicine Philadelphia Penn USA

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Abstract Bipolar electrodes were implanted in the inferior colliculus of 10 healthy guinea pigs and visual detection level thresholds of auditory evoked responses were obtained at 17 frequencies between 0 5 and 20 0 kHz One microvolt isopotential curves from the round window for the same test frequencies were obtained in these animals A comparison between the evoked response threshold curves and previous reports of the behavioral audiogram reveals a favorable correlation The cochlear potential curve however appears to be a less efficient predictor of the audibility curve than the evoked response thresholds The utility of evoked response audiometry from the inferior colliculus for quickly assessing auditory thresholds in the guinea pig is discussed

A great deal is known about the morphology of the auditory system and the physiological mechanisms associated with hearing in the guinea pig Relatively little, however is known about hearing at the behavioral level in this species This problem results from the difficulty in training guinea pigs to respond in the presence of sound The application of classical conditioning (Horton 1933) and Preyer reflex (Gerstner 1942) procedures has met with only limited success Moreover the catatonia like behavior and low food requirements of the guinea pig have precluded many instrumental reward conditioning procedures The recent use of behavioral suppression however, has proved more successful and audibility curves have been obtained using response inhibition to chill induced shivering (Anderson Wedenberg 1965) to lettuce chewing (W Murray, 1966) and to licking behavior (H et al , 1971) Although these procedures been successful in describing the ab threshold of hearing they have required investments of time in training and testing problem of obtaining threshold information rapidly in the guinea pig has severely li the use of this laboratory animal particul a situation in which changes in th sensitivity are important

This study uses an objective physio technique (evoked response audiometry) sess auditory capacity in the guinea pig procedure provides a quick and valid m of threshold sensitivity which may serv valuable alternative to behavioral audi in many experimental situations

METHODS

Subjects and preparation

Ten adult colored guinea pigs weighing tween 200–250 g were used Each animal anesthetized (sodium pentobarbital was administered intraperitoneally) and a tra cannula was inserted A head holder was to secure the animal The soft tissue was vated from the skull from the midline to the external auditory canal The superior b the canal was incised about 3 0 mm la

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ympanic ring The skull was positioned so the foramen ethmoidale and the center of xternal auditory canal were in the horizon lane A small hole was made in the skull 2 mm posterior to the coronal suture and mm to the right of the sagittal suture The was situated directly over the right inferior culus (Rossner 1965)

ater in the experiment the head was rotated and the left auditory bulla was exposed auricularly A small hole was drilled ugh the posterolateral aspect of the bulla to nit access to the round window membrane

ll testing was conducted in a sound at ated booth A 60 W light bulb was placed re the preparation and the ambient air perature was maintained at 37°C

aratus

sine wave output from an oscillator was ied into a tone burst by passing it through lectronic switch The switch was gated at a per second interval to produce 50 msec bursts with a 5 msec rise and decay time tone bursts were amplified and transmitted ı Teledynamics TDH-49 earphone The ulus frequency was monitored with a fre ncy counter and the intensity was set with cade attenuator When the a c cochlear ntial responses were measured the same em was used but the electronic switch was nanently gated so that a tone was presented t nuously

lick stimuli were occasionally used and were produced by passing a 0 1 msec are wave pulse across the earphone

he sound system was formed by sealing the phone against a sound tube which was 4 mm ameter and 35 mm long A 2 mm probe was sed through the center of the sound tube to hin 0 5 mm of the open end The probe tube etrated the sound tube at an angle of 45° ut 20 mm from the open end The tube ended 20 mm farther and was sealed against robe microphone (Bruel & Kjaer 12 5 mm ndenser microphone model 4134) The be tube was calibrated by sealing a Bruel & Kjaer 3 12 mm condenser microphone (model 4138) over the open end of the sound tube in the approximate position of the tympanic membrane of the guinea pig The sound pressure level (SPL) at the probe microphone was corrected for the true SPL assumed to be at the animal s tympanic membrane When the sound tube was positioned in the ear canal it was always placed as close as possible to the tympanic membrane All sound levels detected by the probe microphone were converted to SPL at the tympanic membrane and expressed as dB re 20 $\mu N/m^2$

The signals detected at the electrode were amplified 1 000 times (bandpass 1 Hz to 1 kHz for the responses at the inferior colliculus 100 Hz to 30 kHz for the responses at the round window) The responses at the inferior colliculus were displayed on an oscilloscope the sweep of which was synchronized with the tone bursts The a c cochlear potentials were measured with a frequency sensitive voltmeter (General Radio model 1900A wave analyzer)

Procedures

The responses at the inferior colliculus were monitored first The measurements were made with a bipolar concentric electrode which has a 0 5 mm sleeve and a 0 2 mm center shaft that extended 1 mm beyond the sleeve The epoxy insulation was removed from the end of the sleeve and from the center shaft so that the vertical separation between the recording points was 0 5 mm The electrode was lowered into the brain and auditory evoked responses were monitored as clicks were presented to the ear The final position of the electrode in the inferior colliculus was determined on the basis of the amplitude and latency of the evoked response waveform A response was considered satisfactory if it had an amplitude of 150–200 μV and a latency of 3 5 [illegible] msec to the first negative peak These responses were typically found at an electrode depth of 2 5 3 5 mm below the brain surface The preparation was allowed 10 15 minutes to stabilize before data were collected

A [illegible]

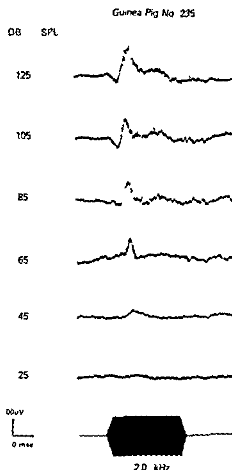


Fig 1 Evoked responses from the inferior colliculus A typical threshold sample for a tone burst in guinea pig No 235 In this example the threshold was about 35 dB SPL

A procedure similar to the method of limits was used to determine a visual detection level (VDL) threshold for the evoked responses at the inferior colliculus Thresholds were obtained at 17 frequencies in successive 1/3 octave steps between 0 5 and 20 0 kHz The SPL of the tone burst was set at a convenient supra threshold level (between 75 and 90 dB) and attenuated in 10 dB steps With each step the oscilloscope was monitored to see if a re sponse could be visually detected This process continued until a SPL was reached which failed to elicit a detectable response The SPL was raised 5 dB and the oscilloscope was moni tored for a response The threshold was defined as the SPL halfway between the highest sound level at which a response was not seen and the lowest sound level at which a response was observed This procedure was repeated at all test frequencies A typical threshold ... seen in Fig 1 for a 2 0 kHz tone burst ... seen, the evoked response am... minishes as the SPL is attenuated ... ample, the threshold was about 35 d... After these thresholds were deter... small lesion was made in the inferior co... by passing a current between the elect... for a few seconds Histologic exam... firmed that the electrode had been ... ferior colliculus

The animal was repositioned and th... window membrane was visualized Th... tube was repositioned, and a small s... electrode, made from 0 175 mm Teflo... silver wire, was placed on the round ... membrane Thresholds for the a c c... potential were determined at each te... quency by adjusting the SPL until a ... response was obtained on the wave ... Data on the cochlear potential were col... 8 animals

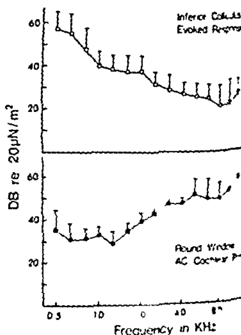


Fig 2 Evoked response thresholds ... lear potential thresh... The ... thresholds at 17 test frequencies (succes... steps) are illustrated in the upper part of the figure ... bars indicate 1 S D above the mean The a c ... µV isopotential curve ... the a c cochlear po... illustrated in the lower part of the figure ... indicate 1 S D above the mean

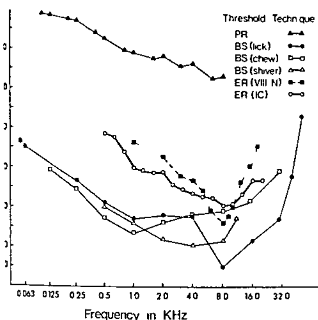


Fig 3 Comparison thresholds by various methods PR Preyer reflex by Gerstner (194?) BS behavioral suppression (lick) by Heffner et al (1971) (chew) by Miller & Murray (1966) and (shiver) by Anderson & Wedenberg (1965) ER evoked response (VIII N) from the eighth nerve of hamster by Finck (196?) and (IC) from the inferior colliculus

RESULTS

ked response thresholds

average VDL thresholds at each test fre ncy are presented in Table I and illustrated ie upper part of Fig 2 The vertical bars in 2 indicate one standard deviation above mean in the 10 subjects Variability was e small and fairly consistent at approxi ely 6 5 dB over all frequencies tested The itest sensitivity is at 8 0 kHz (20 8 dB SPL) re was a low frequency loss in sensitivity ch increased approximately 9 dB/octave be 10 kHz The high frequencies, above 10 0 show a loss in sensitivity of approxi ely 11 dB/octave

cochlear potential thresholds

1 0 μV isopotential thresholds recorded at round window are presented in Table I and are illustrated in the lower part of Fig 2 The vertical bars in Fig 2 indicate 1 S D above the mean The most sensitive frequency tested is 1 25 kHz (28 4 dB SPL) Above this frequency the threshold increases at approximately 8 0 dB/octave

Comparison of threshold methods

The behavioral thresholds reported for the guinea pig are reproduced in Fig 3 The Preyer reflex data reported by Gerstner (1942) demon strate high threshold levels typical of this pro cedure The three absolute thresholds obtained by suppression procedures (Anderson & Wedenberg 1965 Miller & Murray 1966 and Heffner et al 1971) are very similar although Heffner et al (1971) showed greater sensitivity with suppression of licking at 8 0 kHz than was

le I *Mean thresholds (dB SPL) in guinea pigs (frequency in kHz)*

rdi ng	Frequency (kHz)																
	0 5	0 63	0 8	1 0	1 25	1 6	2 0	2 5	3 15	4 0	5 0	6 3	8 0	10 0	1? 5	16 0	20 0
rior lculus	56 3	54 5	47 6	39 8	38 0	37 ?	37 4	31 3	28 3	26 3	25 7	24 1	20 8	21 8	?6 0	33 2	33 2
) ndow	35 3	31 4	31 9	32 7	28 4	34 3	39 5	42 5	47 0	47 0	51 9	49 7	48 6	5? 6	58 3	62 1	62 0

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A Makishima M D
Dept of Otorhinolaryngology and Human Communication
University of Pennsylvania School of Medicine
3400 Spruce Street
Philadel...

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NORMAL CRITICAL BANDS IN THE CAT

J O Pickles

From the Neurocommunications Research Unit The Medical School Birmingham England

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Critical bands in the cat were measured by a oural psychophysical method Pure tones were noise of variable bandwidth but constant total geometrically centred on the test tone, the point at the masked threshold began to fall as the masker dth was increased estimated the critical band At 2 kHz the critical bandwidth was also measured wideband masked thresholds of both tones and f variable bandwidth this produced the same result first method The measured critical bandwidth was r than previously published values of the effective idths of single fibres of the auditory nerve The do not fit in with the commonly accepted theory e critical band represents the resolution of the coch

an psychophysical experiments with lex stimuli have shown that at any one ency the auditory system possesses a defined bandwidth of resolution This width is known as the critical bandwidth s constant over many different auditory (Feldtkeller & Zwicker, 1967, Scharf, The same critical bandwidth is for in e thought to govern the masking of sig by broadband noise, the thresholds and ved loudness of complex sounds, and attainable limits of both frequency and oral resolution (Scharf, 1970) It is gener accepted, although only on indirect evi e, that the mechanism of critical bands is ted in the cochlea itself, and that the criti bandwidth equals the bandwidth of the anical resolution of tones in the cochlea ever there has hitherto been no direct test, as critical bands have not been measured directly in any species for which measures of the resolution bandwidth of the various stages of the auditory system have been available The mechanical resolution of the cochlea has in any case only been measured at high sound intensities where, in man at least, the critical band becomes very broad If critical bands were indeed set up in the cochlea, one might expect the critical bandwidth to be reflected in the resolution bandwidth of the cochlear out put, that is in the bandwidths of single fibres of the auditory nerve Their effective band widths, or bandwidths of their equivalent rec-tangular filters should be equal to the effec tive bandwidth of the critical band measured at the same intensity and frequency In the ex periments described here, critical bands have been measured in the cat by a behavioural psychophysical technique As a direct test of the cochlear origin of critical bands the band widths were compared with the effective bandwidths of single fibres of the auditory nerve, measured by Evans & Wilson (1971)

Work was supported by a grant from the Science rch Council of the U K

METHODS

Estimation of critical bandwidth

A pure tone stimulus was masked by a noise band of constant total power but variable bandwidth, geometrically centred on the test tone Pure tone maskers were not used as the auditory nerve does not necessarily behave linearly to combinations of pure tones even at

threshold, whereas it seems to do so for broadband stimuli at low intensities (Sachs & Kiang, 1968; Evans & Wilson, 1971) For masker bandwidths smaller than the critical band the masked threshold should be independent of masker bandwidth if the results of Greenwood (1961 *a*) apply, and for masker bandwidths larger than the critical band the masked threshold should decline in inverse proportion to masker bandwidth, that is, at 3 dB per octave of masker bandwidth This model was tested and found to be adequate (Results section). The relation between the masked tone threshold and masker bandwidth both on logarithmic scales, was therefore fitted with a function of two intersecting straight lines, one of zero slope for small bandwidths, and one of slope −3 dB per octave for larger bandwidths, using the method of least squares The intersection was at the estimated critical bandwidth Although the method is only strictly applicable if the critical band function is rectangular in shape, it in fact closely estimates the effective bandwidth of the critical band over a wide variety of shapes If for instance the critical band function is of Gaussian form, the method produces a value of the effective bandwidth which is overestimated by between 10 and 15%, for the bandwidths actually used

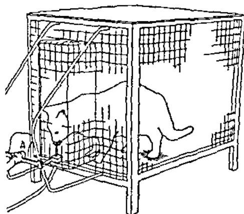
Stimuli

The output of a Solartron type CO546 oscillator was gated with an FET gate giving a signal which was a series of tone pips each of rise and fall time 22 msec, duration 169 msec, and repeated at 3/sec Bandpass masking noise was produced by modulating lowpass filtered thermal noise (low frequency cutoff 20 Hz) with a carrier sinusoid in a balanced modulator The high frequency cutoff slope of the lowpass filter was 12 dB per octave The slopes of the output band of noise depended on the modulation frequency and the noise bandwidth for instance the lowpass cutoff slope modulated in frequency to ten times its original frequency will become a cutoff of 120 dB per octave All harmonics in the [illegible] band were 60 dB or more below [illegible] band, except the third harmon[illegible] 39 dB below An attenuator ganged [illegible] pass filter cutoff control ensured th[illegible] power to the modulator stage wa[illegible] within ±0 2 dB whatever the band[illegible] accuracy of the final output band [illegible] was checked in three ways (i) Th[illegible] noise spectrum was checked in [illegible] bands (Brüel & Kjær bandpass fi[illegible] 1612) (ii) The output of the bala[illegible] ulator stage was checked with a w[illegible] ser with 10 Hz resolution (Feder[illegible] fic 'Ubiquitous Spectrum Analy[illegible] The technique of estimating the effe[illegible] width of a filter by the method [illegible] above for measuring critical ba[illegible] applied to measuring the bandwid[illegible] of known width The total noise [illegible] was 30 dB SPL at 1 and 2 kH[illegible] dB SPL at 8 and 16 kHz The hig[illegible] sity was used at the high frequenc[illegible] cat's tongue, in the licking task u[illegible] generated some noise

One of three transducers and amp[illegible] used, depending on the test freque[illegible] At 2 kHz and below, a cone speaker [illegible] adequate A Goodmans Axiom sp[illegible] used, driven by a Leak TL/12 powe[illegible] followed by a 40 dB power atten[illegible] kHz an Ionofane ionic speaker was [illegible] 16 kHz a type 4132 [illegible] inch Brüel [illegible] microphone with grid removed was [illegible] sound source, with 200 V polarisatio[illegible] en at 2 V RMS maximum Sound fi[illegible] calibrated with Brüel & Kjær half [illegible] quarter-inch condenser micropho[illegible] 4133 and 4135, grids removed) and [illegible] Kjær type 2606 Precision Sound Le[illegible]

Measurement of thresholds

Cats were tested in a sound proof r[illegible] sound absorbent inner surfaces A [illegible] shuttle box was found to be satisf[illegible] measuring thresholds at 2 kHz and be[illegible] box was 60 cm high 60 cm wide an[illegible]



Apparatus for the measurement of thresholds at requencies The sound transducer is mounted at *A* ie cat licks a small dish for milk which is supplied th a tube Wire baffles in the cage ensure that the cat with its head in only one position Stimuli are only ited while the cat is licking and so its head is in the sound field

divided in two by a barrier 4 cm high floor, through which shocks were given, isted of bars 1 7 cm apart Alternate bars connected to one polarity of the shock rator The current applied to the cat was lly 3 mA, although because the bars were ose together most of this probably flowed ss the surface of the foot pads The ker was mounted axially 2 m above the The warning period was 15 sec long and re to detect was punished on a variable schedule, the ratio varying from 0 5 to depending on the cat
ove 2 kHz, the free field method was d to be completely inadequate in most of essions the responses were too inconsist to allow a threshold to be assessed, and n one could it was much higher than exed Instead conditioned avoidance during rg was used Stimuli were only delivered e the cat was licking for milk, and so its l was fixed in front of the high frequency sducer (Fig 1) The field was even to ±2 ver the space occupied by the cat's head over the one-octave band (at 8 kHz) or ave band (at 16 kHz) containing the nh Wire mesh barriers in the cage ensured that the cat could lick only with its head in one position The cats were food deprived and fed entirely on the 120 ml of evaporated milk (Carnation Milk, Carnation Foods Co Ltd, England) obtained each day in the apparatus Licking the dish, which was 2 cm diameter, initiated a trial Once the trial had begun milk was pumped into the dish at the rate of 3 ml/min while the cat was licking On 1/3 of trials the stimulus was switched on after 5 sec if the licking was steady In this case the first lick between the 15th and 20th sec of the trial was punished with a shock on a variable ratio schedule, the ratio used varying from 1 0 to 0 3 depending on the cat Cessation of licking in the danger period showed that the cat had heard the stimulus The other 2/3 of trials were dummies and used to assess the false positive detection rate Thresholds were measured by tracking with a final step size of 1 dB as described by Pickles & Comis (1973) The criteria they described for an equilibrium threshold series were used here, with one exception When measured by the licking technique some animals thresholds fell steadily throughout the session presumably due to declining hunger In these cases the threshold was assessed over a fixed number of trials in the session

Subjects

All cats were monaural the contralateral middle ear apparatus having been destroyed and the middle ear cavity filled with bone cement All except cat 32 had cannulae implanted over the operative cochlear nucleus at some time during testing as described by Pickles & Comis (1973) In all 76% of thresholds were obtained in cats after implantation Measurements before and after the implantation as described in the results section showed that cannulation did not in fact affect the critical bandwidth The cats absolute thresholds were comparable with those previously described (Elliott et al, 1960, Neff & Hind, 1955, Igarashi et al, 1972)

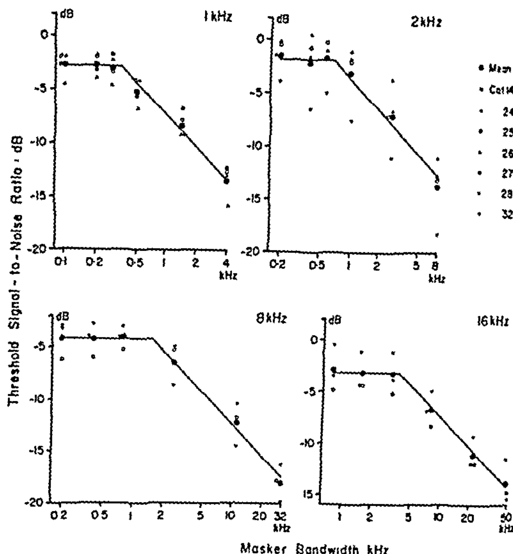
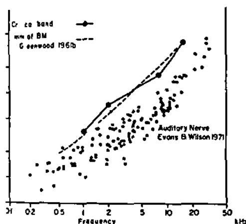


Fig 2 Masked tone thresholds for different bandwidths of masking noise at the four test frequencies The masking noise is of constant power but variable bandwidth geometrically centred on the test frequency The mean thresholds have been fitted by lines of zero slope −3 dB per octave The corner is at the critical bandwidth

RESULTS

Fig 2 shows each cat s mean thresholds in all bandwidths of noise for the four test frequencies The mean thresholds taken over all the cats have been fitted with the expected two-part function, namely, a line of zero slope inside the critical bandwidth and one of slope −3 dB per octave outside the critical bandwidth Around these mean values considerable scatter of individual cats thresholds is apparent Closer inspection however shows that each cat s thresholds are much more consistent within themselves than this scatter might at first suggest For instan thresholds of cat 32 at 16 kHz are con higher than the mean but are well fit two-part function similar to that fitted mean Presumably this cat has a critical width similar to the mean but a threshold signal-to-noise ratio Simil 25 at 16 kHz has a threshold which is c ently lower than the mean but which fitted by a two part function of approx the same critical bandwidth Between cats there may be a range in estimated bandwidth rather than in threshold se



3 The critical bandwidth at the four test frequencies critical bandwidth is clearly greater than the effective widths of f bres of the cat auditory nerve which were mined by comparing the fibres thresholds to tones their thresholds to wideband noise (Evans & on 1971) However the critical bandwidth cor closely to 1 mm of the cat basilar membrane as ted by Greenwood (1961 *b*)

e ratio The greatest range is shown at 8 where the thresholds of cat 28 are closely d by a two-part function giving a critical dwidth of only a quarter of that of cat 25 same population of cats was not neces ly used for each test frequency, compari between frequencies must therefore be le with caution

/ariability of the sort described above will e only a small effect on the fit of the mean sholds to the expected two part function he fit of the data to the function can be wn to be adequate, then the method can be ected to be working and the position of the ner can be used to estimate critical band lth Different functions might however e been expected, in particular slopes of not o but −1 5 dB per octave have been some es reported for the relation between eshold and bandwidth within the critical d (Hamilton 1957) In order to test this ssibility, two-part regression lines were fit by the method of least squares The mean pe for narrow bandwidths was −0 07 dB per ve (S D 0 5 dB per octave 10 d f) This s not significantly different from zero slope but was significantly different from a slope of −1 5 dB per octave ($p<0\ 05$ 2 tailed) For wide bandwidths the slope also agrees with the value expected, the mean fitted slope was −3 04 dB per octave (S D 0 16, d f =15), not significantly different from the expected −3 dB per octave The expected function moreover, provides an adequate account of the variance in the original data The variance of each point fitted calculated from the var iance within the raw data, was 0 274 (dB scale 104 d f), once the mean of each cat s thresholds was adjusted to the same grand mean That expected from the variance of the mean points around the fitted lines was 0 247 (16 d f) The difference is not significant (*F* test) The origial two part function seems therefore to be an adequate description of the data and the position of the corner can be expected to estimate the critical bandwidth

Fig 3 shows the value of the critical band width as a function of test frequency The standard deviation of each value calculated from the fit of the data to the expected two part function is between 5 and 15% other unknown errors may of course be larger As a function of test frequency, the critical band width increases with a mean slope of 0 80 when plotted on log log axes Fig 3 also shows the relation between the measured criti cal bandwidth and two measures of cochlear function The critical bandwidth is clearly greater than the mean of the effective band widths of single auditory nerve fibres Direct comparison is possible as the measures are made in a way which is substantially independ ent of the shape of both the tuning curve and the critical band resolution function The difference is approximately 2 5 times and is constant over the whole frequency range Furthermore intriguingly the cat critical band width corresponds closely to the 1 mm of basilar membrane originally suggested by Greenwood (Greenwood 1961*b*)

One objection to comparing effective band widths of auditory nerve fibres with those of the critical band is that the two were measured

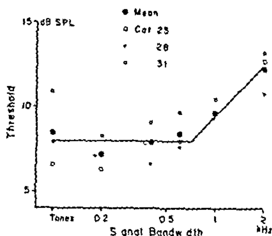


Fig 4 Wideband masked thresholds of both tones and noise bands centred on 2 kHz The noise signal is of constant power but variable bandwidth the tone threshold is the mean of the thresholds of five tone signals evenly spaced between 1 70 and 2 30 kHz The mean thresholds have been fitted by one line of zero slope and another of slope +3 dB/octave The corner is at the estimated critical bandwidth which is very close to that estimated from the data in Fig 2

at different intensities and by different methods Evans & Wilson (1971) measured the fibres effective bandwidths by comparing their absolute thresholds to pure tones with their absolute thresholds to wideband noise Here masked tone thresholds were measured in different bandwidths of masking noise at 30 to 50 dB above the cats absolute threshold An attempt was therefore made to measure critical bands by a method corresponding to that of Evans & Wilson, namely by comparing the absolute thresholds of tones and of noise of different bandwidths When used psychophysically however this method will only work if used at the most sensitive part of the audiogram There is a danger that otherwise as the signal bandwidth is widened the signal will be detected in a different frequency range where the animal is more sensitive As this was not necessarily possible the audiogram was flattened by wideband masking noise at an intensity which in fact raised thresholds from a mean of −14 dB SPL to +8 dB SPL The masking noise was of constant power per octave and the noise signals were centred arithmetically at 2 kHz Five frequencies of pure tone signal were used spaced [illegible] within the expected critical band at [illegible] namely at 1 70, 1 85, 2 0 2 15 and 2 3 [illegible] Fig 4 shows the relation between [illegible] and stimulus bandwidth A two-part [illegible] of slopes zero and +3 dB per octave [illegible] fitted to the data estimates the critical b[illegible] width at 708±75 Hz, in close agreement [illegible] the value of 700 Hz±99Hz obtained [illegible] earlier method This suggests that the [illegible] bandwidth does not change with [illegible] moreover that it seems to be the same [illegible] measured by the spectral resolution of [illegible] rowband stimulus in wideband masking [illegible] or by the spectral integration of a wide[illegible] stimulus Similar behaviour has been [illegible] in human beings (Gässler, 1954 Fel[illegible] Zwicker 1967) Direct comparison be[illegible] the neural and psychophysical data is [illegible] fore possible

The overall mean determinations are [illegible] in Table I

At some time during testing eight [illegible] nine cats used had cannulae implanted [illegible] the operative cochlear nucleus as des[illegible] by Pickles & Comis (1973) Drugs [illegible] affect specifically the centrifugal [illegible] of the cochlear nucleus, were applied [illegible] surface of the cochlear nucleus in [illegible] study the influence of the centrifugal [illegible] tion on the critical bandwidth These [illegible] will be described separately The [illegible] after-effect of cannulation and injection [illegible] critical bandwidth was assessed by com[illegible] the critical bandwidth at 1 kHz before [illegible] after all intervention The *normal* [illegible]

Table I *Summary results of the critical [illegible] width critical ratio and signal [illegible] within the critical band*

Frequency kHz	1	[illegible]	[illegible]	[illegible]
Critical bandwidth kHz	0 35	0 70	1 [illegible]	[illegible]
10 log critical bandwidth	[illegible] 4	[illegible]	31 8	[illegible]
Critical ratio dB	[illegible] 6	[illegible]	[illegible]	[illegible]
Signal to Noise ratio dB	[illegible] 8	[illegible]	4 [illegible]	[illegible]

dwidth was 345 Hz±0 076 log units (4 d f) that after all intervention was 371 0 045 log units (4 d f) The increase is 2 log units which is not statistically ificant If the difference between the al and psychophysical resolution band- hs were to be ascribed to cannulation, the ulation would have to have been having ffect which, on the basis of the above data, ld be expected to arise with a probability ss than 0 0005 (1 tailed) It is very unlikely efore, that the observed difference be en the neural and psychophysical resolu bandwidths was due to the cats' being ormal

DISCUSSION

rious measurements of the cat's sensitivity ignals in noise have been confined to de inations of the masked threshold in bands asking noise wider than the critical band tson 1963) This produces a measure ch has been called the critical ratio icker et al , 1957) and is calculated as the er of the signal at masked threshold minus power per unit bandwidth of the masking e The critical ratio can be calculated in present experiment from the data in Figs 2 4 and is shown in Table I At 1 and 2 kHz verages 1 5 dB greater than that obtained Watson, and at 8 and 16 kHz it averages 4 less The discrepancy at the higher fre ncies may be caused by differences in nique In the present experiment especial e was taken that the sound field should be n at these frequencies and that the cat s d should be in a constant position enever the stimuli were presented A free d shuttle box and cone speakers as used Watson (1963) produced much higher esholds at the high frequencies In fact, tson made some measurements with an nc loudspeaker, which should have pro- ced a more even sound field He obtained esholds which were closer to the present es but decided to ignore them as they did not agree with his other measurements They may, after all, have been the more accurate

The sensitivity to signals in wideband noise is determined by two factors One is the bandwidth of the critical band filter, or in other words the noise power from which the signal must be discriminated after filtering by the critical band mechanism The other factor is the signal to-noise ratio at which the signal can be detected in the resulting band of noise In man, the signal to noise ratio within the critical band is commonly thought to be −4 dB above 1 kHz (e g Scharf, 1970), although not all measurements agree with this (Greenwood 1961 *a*) In the present experiments it is a little greater, with a mean value close to −3 dB

A second factor which in man is thought to be related to the critical bandwidth is frequency discrimination it has been suggested that the frequency discrimination limen is proportional to the critical bandwidth (Fletcher, 1940, Zwicker et al , 1957) The present re sults, together with the frequency discrimina tion data of Elliott et al (1960) suggest that this may not be so in the cat The measured critical bandwidth is 40 times the frequency limen at 1 kHz 49 times at 2 kHz, but declines to only 16 times at 16 kHz There is similar lack of proportion between the cat frequency discrimination threshold and the auditory nerve fibre effective bandwidths Lack of proportion between frequency discrimination and the critical ratio has also been found above 1 kHz in the parakeet (Dooling & Saunders 1975) Moore (1974) using a frequency dis crimination method which reduces intensity cues, has found similar results in man The generally accepted proportionality in man may not therefore be of fundamental significance

A third measure which in man is believed to be related to the critical bandwidth is position on the basilar membrane It was suggested originally by Fletcher (Fletcher, 1940) and la ter by Greenwood (1961 *b*) that frequencies separated by a critical bandwidth were rep resented a constant distance apart on the basi- lar membrane Greenwood, on the basis of

Bekésy's experiments (Bekesy, 1960), showed that this was so in man, and that the distance was 1 mm He suggested that this would be true in other species This in fact seems to be true for the cat (Fig 3) Whether it is just a coincidence or stems directly from the mechanism of the critical band will be discussed below Fletcher's view, and one that has been generally held since, was that the critical bandwidth represented the mechanical frequency resolution of the cochlea The relation between the critical band and constant distances of the basilar membrane supported this view, although it was contradicted by Bekesy's measurements on human cadavers which showed that the mechanical frequency resolution of the cochlea was up to six times poorer than that of the critical band (Bekésy, 1960) Recent measurements have shown that the basilar membrane is in fact much more sharply tuned than previously thought (Rhode, 1971) However, measurements of the basilar membrane are not yet reliable enough at medium and low intensities to make comparison with the critical band worthwhile A second measure of cochlear frequency resolution which can be obtained under rather more physiological conditions is the frequency response of single fibres of the auditory nerve Two components of the frequency response can be identified A sharply frequency selective response is found in what has been called the 'tip' of the tuning curve, namely, within an intensity range of about 40 dB above the fibre's lowest threshold and over a narrow frequency range around it Within this range the fibre seems generally to behave linearly to broadband stimuli and hence is of constant effective bandwidth from threshold to about the 40 dB limit above it (Evans & Wilson, 1971, de Boer, 1969, Moller, 1970, de Boer, 1971) At higher intensities in the 'tail' of the tuning curve, the effective bandwidth becomes much greater (Kiang et al , 1965) In human beings the critical band correlates with this behaviour, although the intensity ranges are different From threshold to some 70 dB above the critical band is of constant wid[illegible] 1954, Feldtkeller & Zwicker, 196[illegible] intensities the critical band seems to [illegible] siderably wider If therefore compar[illegible] be made between the critical band[illegible] the auditory nerve fibre effective band[illegible] might be appropriate to begin by comp[illegible] the low intensity critical bandwidth [illegible] effective bandwidth of the sharply-tun[illegible] the tuning curve Such a comparison [illegible] in Fig 3 and shows that the critical band[illegible] is in fact greater In fact the discrepanc[illegible] be greater than is immediately appar[illegible] critical bandwidth in this task can be exp[illegible] to be governed by the fibres that ar[illegible] sensitive to masked tones Those fibres [illegible] the absence of other factors, be the f[illegible] the narrowest effective bandwidths F[illegible] shows that the critical bandwidth is ap[illegible] mately four times greater than the e[illegible] bandwidth of the narrowest neurone[illegible] does the bandwidth of the broad[illegible] high intensity, tail of the tuning curve [illegible] critical bandwidth Although values of [illegible] fective bandwidth of the tail have not [illegible] published, the data of Kiang & Moxon [illegible] suggests that the 3 dB bandwidth of the [illegible] dip in the tail can be as wide as its [illegible] frequency This is several times great[illegible] the critical band on logarithmic scales [illegible] further difficulty arises from the obser[illegible] of Watson (1963) that the rat[illegible] signals in noise is constant over a 60 dB [illegible] above absolute threshold, this range [illegible] span both the sharply-tuned and broad[illegible] portions of the tuning curve

Some nonlinear effects with broad[illegible] stimuli have been described in the [illegible] monkey auditory nerve by Ruggero (19[illegible] found that widening a band of noise [illegible] neurone's characteristic frequency [illegible] suppress its responsiveness If such a [illegible] were to determine thresholds in the p[illegible] measurements, one might expect the dec[illegible] masked thresholds to be different fr[illegible] per octave for maskers wider than the cr[illegible] band This is not so in the present exper[illegible]

has it been described in man If the supsion occurred with noise bands narrower the critical band, one might not ex- the threshold of a complex stimulus to ndependent of its bandwidth, if its width arrower than the critical band Such in ndence has been shown in human beings Gassler (1954) The evidence at present s not support nonlinear mechanisms in the tory nerve as being the basis of the critical dwidth, although further experiments may nge this

the discrepancy between the critical dwidth and the bandwidth of the auditory e fibres indeed exists, it might be sug-d that it arises from the different condi s in which the observations were made fibres were recorded in the anaesthetized whereas the critical bandwidth was meas-l in the unanaesthetized, attentive, animal activity of the olivocochlear bundle is ight to be too low in even the decerebrate to affect the afferent fibres (Fex, 1965), reas it might be greater in the intact, un-esthetized cat It is known that its activity increase the bandwidth of auditory nerve s by selectively inhibiting the sharply d tip (Wiederhold, 1970) However lesions he crossed component of the olivocochlear dle do not seem to affect the cat's ability to ct signals in noise, certainly they do not rove it (Galambos, 1960, Trahiotis & El l 1970, Igarashi et al , 1972) The activity the middle ear muscles in the un esthetized cat might also, by changing the uency response of the middle ear, con ably increase the minimum measurable re ution bandwidth of the auditory system wever that the resolution, as shown by the itory nerve fibre bandwidths, does not in t vary with anaesthetic is suggested by mons & Linehan (1968) They recorded at they thought was the same fibre in both anaesthetized and unanaesthetized cat and nd very much the same bandwidth

These facts and the present observations st doubt on the simple identification of criti cal bandwidth with auditory nerve resolution, and hence with cochlear resolution This follows, for if the mechanical resolution of the cochlea indeed determined the critical bandwidth, correlates should be observable in the responses of the auditory nerve, as it is those that influence the higher centres The correlation of the critical bandwidth with 1 mm of basilar membrane may therefore just be a coincidence Two alternative hypotheses are possible One is that the resolution of the coch lea after all determines the critical bandwidth, but that the unanaesthetized, attentive, animal uses in the discrimination of complex stimuli some properties of the auditory nerve which are different from those currently understood Another hypothesis is that the critical band width reflects integrative processes at higher centres Whether or not the critical bandwidth can be identified with processes in the cochlea, or indeed with any one stage of the audi tory system can only be answered by further physiological and psychophysical investigations in animals

ZUSAMMENFASSUNG

Die Breite der Frequenzgruppen bei der Katze wurde durch eine psychophysikalische Verhaltensmethode gemessen Reine Tone wurden durch Geräusche von variierender Bandweite aber konstantem geometrisch auf den Prufton zentriertem Totalschalldruck verdeckt Der kritische Frequenzabstand wurde durch den Punkt bestimmt an dem die Prüfschwelle niedriger wurde wahrend die Verdeckungsbandweite erhöht wurde Bei 2 kHz wurde der kritische Frequenzabstand auch durch die durch Weitband verdeckte Prüfschwelle für Töne and Gerausche von variierender Bandweite bestimmt Dies ergab ein mit der ersten Methode übereinstimmendes Resultat Der auf diese Weise bestimmte kritische Fre quenzabstand war grösser als die bisher publizierten Werte fur effektive Bandweiten von Einzelfasern des Gehörnerven Die Resultate stimmen daher mit der allge mein akzeptierten Theorie nicht überein dass der kritische Frequenzabstand die Resolution der Cochlea repräsentiert

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J O Pickles Ph D
Neurocommunications Research Unit
The Medical School
Birmingham B15 2TJ
England

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ACTIVE OTOSCLEROTIC FOCI IN THE STAPES

An Electron Microscopic Study

D J Lim and W H Saunders

From the Otological Research Laboratories Department of Otolaryngology The Ohio State University College of Medicine Columbus Ohio USA

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ract A total of 12 undecalcified and decalcified oto -otic stapes containing active spongiotic lesions were mined using an electron microscope Evidence of olytic osteolysis was seen in all specimens but oclasts were observed in only four The advancing in the spongiotic lesion was 'moth eaten due to the ineralization of canaliculi and lacunae The de eralizing process appeared to be initiated by degranula of lysosomes by the degenerating osteocytes As bone ept on continued poorly mineralized new bone was down by osteoblasts which showed mitochondrial lling The possible implication of this mitochondrial ll ng is discussed in relation to the pathology of the sclerosis

nsiderable interest has been raised in recent rs concerning the basic mechanisms in- ved in bone destruction and remodeling by otosclerotic foci (Rüedi, 1969, Chevance et , 1969, Clarke, 1969) Chevance et al (1970) posed that the resorption of bone in the sclerosis was mainly mediated by osteocy- osteolysis in which lysosomes containing d hydrolases were released into the bone trix This process presumably led to the mineralization and remodeling of the ground bstance that was controlled by the bone cells ese bone cells were inferred to be genetically normal in otosclerosis, and when new bone is laid down by these cells, they produced normal ground substances which either failed to mineralize completely or developed sclerotic bone with an abnormal crystalline formation

Many investigators attempted to study the demineralization and remineralization of the otosclerotic bone using the X ray diffraction technique (Meurman et al , 1967 Puhakka, 1971), microradiogram (Clarke, 1964) and tetracycline labeling method (Rockert et al 1965 Roberto et al , 1973) However the in formation as to the cellular involvement leading to the pathological events was limited owing to the difficulty of preparing mineralized speci mens for an ultrastructural investigation

This study was undertaken in order to eluci- date the fine structures of the bone cells and to shed light on the dynamics of mineralization in active otosclerotic foci in both undecalcified and decalcified otosclerotic stapes

MATERIALS AND METHODS

A total of 12 otosclerotic stapes with active otosclerotic lesions obtained from patients who underwent surgery was examined Three per- cent cacodylate buffered glutaraldehyde (pH 7 2–7 4) was used as an overnight fixative, and the specimens were post fixed in cold 1 3% s-collidine buffered osmic acid (pH 7 2–7 4) for 1 hour Six specimens were decalcified in 4 5% EDTA for 1–4 days and washed in Tyrode's

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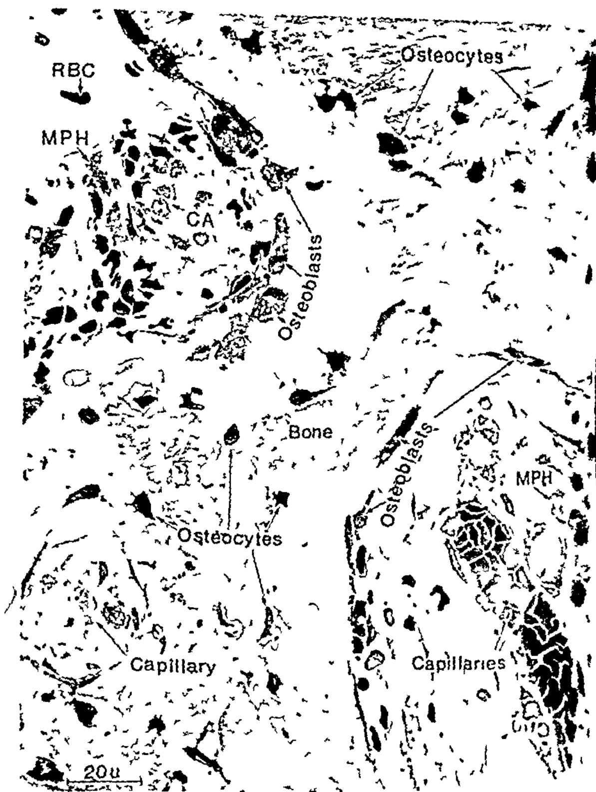
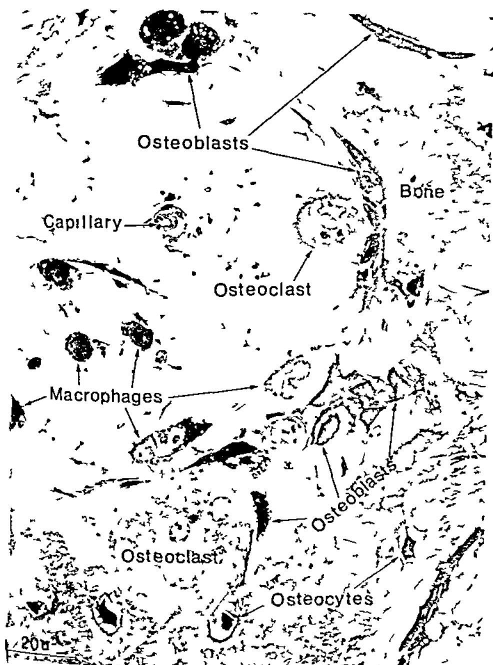


Fig 1 A low-power electron m crograph of an active otosclerotic lesion shows enlarged marrow spaces conta n ing osteoblasts macrophages (*MPH*) RBC and blood capillaries (*CA*) Numerous steocytes occupy the lacunae Decalcif ed

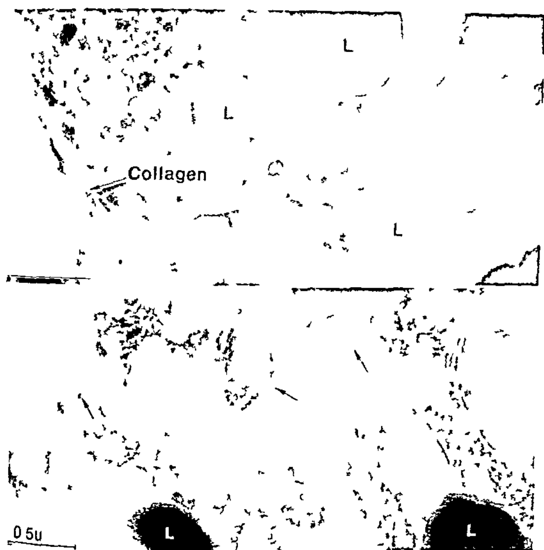


2 An overall view of the active lesion shows numerous -oblasts macrophages and osteoclasts Decalcified

-tion for 1 hour before being embedded in -on as described earlier (Lim 1970) and the -aining 6 specimens were embedded in hard -oxy resin (Epon 812) without decalcification -th groups of specimens were thick sectioned with a glass knife for phase-contrast microscopy and thin sectioned with a diamond knife for electron microscopy Silver or gold colored sections were then placed on a carbon-coated formvar membrane and



Fig 3 (A) A ... demarcati... mineralize... ... osteocytes (*arrows*) *L* a lacuna with primitive collagen Undecalcified (B) An example of clear delineation between mineralized bone and unmineralized collagen fibers in the advancing front of an active otosclerotic lesion A dissolving lacuna (*L*) with numerous dense granule... (*arrows*) is show... calcified (C) An active le... n shows a ... pearance caused by demine... along the ... canaliculi A degenerating osteocyte (*Oc*) n ... lacuna Collagen fibers ( ) are exposed in ... area Arrows point to ... spheri... Undecalcified



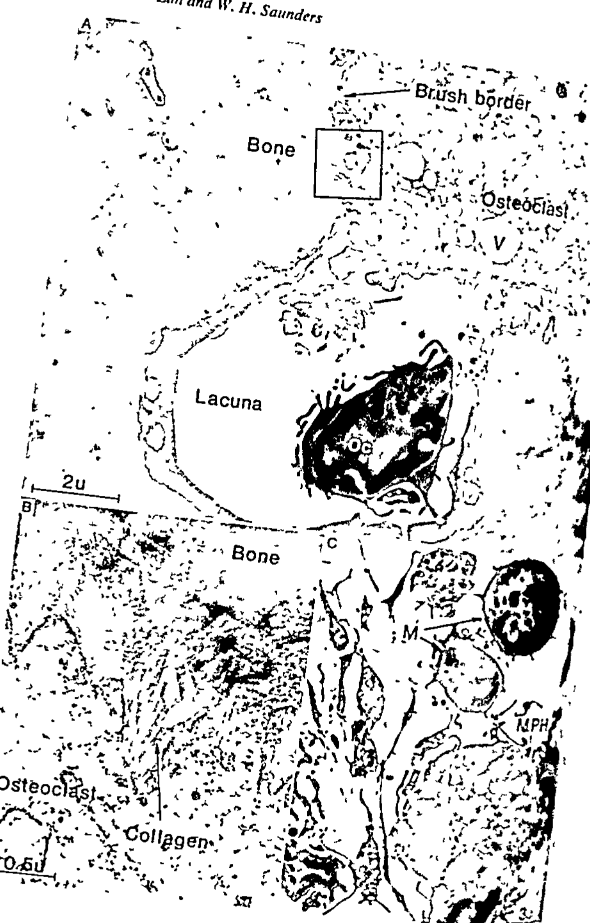
1 (A) A resorbing lacuna of an active lesion contains numerous lysosomal granules presumably from degenerating osteocytes. Undecalcified. (B) A close-up view of the margin of a resorbing lacunar wall shows numerous apatite crystallites (*arrows*). Dark bodies are degranulated lysosomes. Undecalcified.

ble stained with uranyl acetate and lead ite. The sections were then examined under nsmission microscope (Philips EM300) us a magnification range between 220× and 000×

OBSERVATION

stapedial bones with active otosclerotic ons were spongious due to an extensive pillary infiltration and enlarged marrow spaces filled with loose connective tissue. Both the bony resorption and new bone formation were evidenced by a large number of osteocytes, osteoclasts, macrophages and osteoblasts in the same specimen (Figs. 1 and 2). Lacunae were occupied with osteocytes in the active lesions, in contrast to the normal stapes where the lacunae were usually empty (Fig. 1).

When mineralized otosclerotic foci were examined, demineralized demarcation was clear


A
Brush border
Bone
Osteoclast
V
Lacuna
OC
2u
B
Bone
C
M
MPH
Osteoclast
Collagen
0.5u

on an ultrastructural level in cases where
bone appeared to be dense (Fig 3 A and B)
n other cases, the advancing front showed
th-eaten appearance caused by the resorp-
of lacunar walls and canaliculi, and by the
adic residual calcified islands of ground
tances among demineralized collagen fi-
(Fig 3 C) In these resorbing lacunar
es, degenerating *osteocytes* and numerous
anulated lysosomes were often seen in
rent stages of the progression of degranula-
and demineralization (Fig 4 A and B) In a
magnification view, bone–apatite crystal
were loosened at the demineralizing front
out osteoclasts (Fig 4 A)

addition to numerous degenerating
ocytes, *osteoclasts* were also observed in
numbers in the area of bone resorption in
t of the 12 spongious bones examined (Fig
n one case, an osteoclast was destroying
d tissue including an old lacuna (Fig
The osteoclast, in general, was multi-
eated and possessed a large cytoplasm
h contained numerous mitochon-
, ribosomes vacuoles and Golgi s ap-
tus A microvilli like brush border made
contact with the bony surface which
wed evidence of demineralization with ex-
bone collagen fibers (Fig 5 B) Crystal-
resembling bone apatite were observed
een the borders of cytoplasmic processes
in vacuoles of the osteoclast, indicating an
ve role of the osteoclast in the demineraliz-
of bone

Macrophages were also seen in large
nbers in marrow spaces, particularly where
ive bone resorption was taking place In
these cells, we observed numerous cyto-plasmic inclusions such as lysosomal granules and lipid droplets and well-developed pinocytotic vesicles indicating active phagocytosis (Fig 5 C) In some instances, crystallites were found near macrophages in the marrow spaces, although it was not certain whether the crystallites were taken up by these cells There were also numerous monocytes in the marrow space giving the impression that macrophages might have originated from them

As the bone destruction continued, small capillaries were budding into the marrow spaces (Fig 2), giving the appearance that osteolysis preceded the capillary infiltration In some areas adjacent to an active lesion, evidence of capillary degeneration was seen in the marrow space (Fig 6), where only scanty connective tissue and few cells were observed Still, whether the capillary degeneration preceded the more active cell infiltration could not be determined As the number and size of blood capillaries increased, the *osteoblasts* became more numerous, and they were lined up along the osteoid surface facing the marrow cavity (Figs 1 and 2) They showed a slender cell body with a dense cytoplasm due to its rich ribosome contents Rough endoplasmic reticulum was extensively engorged, and Golgi's apparatus was well developed indicating active cell function Numerous finger like cyto-plasmic processes made contact with those of neighboring osteoblasts (Fig 7) The cytoplasmic processes were also embedded in the loose collagen layers immediately adjacent to the bone These collagen fibers appeared to have been freshly laid down by the osteoblast and were not mineralized in these active otosclerotic regions The osteoblasts that produced non mineralized collagen fibers showed severe mitochondrial swelling (Fig 6 A) similar to the swelling in the osteoblasts of experimentally induced atrophic rhinitis (Fetter & Capen, 1971) Precollagen fibrils were abundant in the area immediately adjacent to the oesteoblasts, and they then appeared to reaggregate forming mature collagen fibers (Fig 7 B)

5 (A) A close-up view of an osteoclast actively en
in bone resorption Part of the lacuna is destroyed by
osteoclast A small osteocyte (*Oc*) is seen in a large
ially destroyed lacuna *V* vacuole The square is
gnified in (B) Decalcified (B) A high power view of a
sh border reveals that collagen fibers of the bone are
osed by the osteoclast Decalcified (C) A macrophage
(*M*) containing numerous lipofuscin and lysosomal
nules is seen in the marrow space of an active lesion *M*
nocytes Decalcified

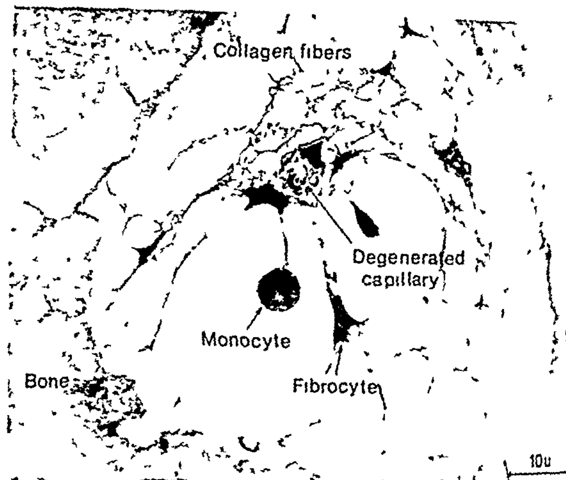
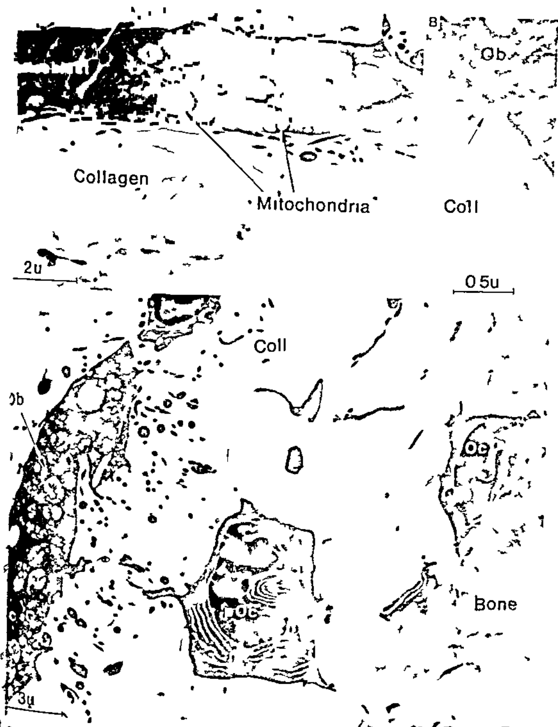


Fig 6 A marrow space closer to an active lesion shows a degenerating capillary (*arrow*) and a paucity of connective tissue and cells Decalcified

As an osteoblast became completely isolated by newly laid down collagen fibers this isolated cell became a preosteocyte which still maintained the structural characteristics of the osteoblast such as a large cytoplasm–nuclear ratio a well-developed endoplasmic reticulum (ER) and numerous mitochondria (Figs 7C and 8A) In these preosteocytes lacunar walls were rough and surrounded by a large amount of unmineralized collagen fibers lacking the Rouget Newman zone (Fig 8A) When the lacunar wall was mineralized a typical Rouget Newman zone formed by dense unmineralized ground substance could be recognized (Fig 8B) The *osteocytes* occupying the lacunae in newly formed otosclerotic bone often possessed infolded nuclei and abundant ER This appearance was in contrast to the osteocytes found in demineralizing old … the advancing front of the otoscleros… had large round nuclei and a small … cytoplasm with numerous lysosomal … (Fig 8C)

Ground substances of newly form… sclerotic bone were made mainly of … collagen fibers and amorphous grou… stance that became mineralized Co… fibers in the old lacunae undergoing re… contained broad collagen fibrils … form) as reported by Reydon & S… In the early stage of calcification there … numerous calcospherites which were f… in association with nucleation centers … to those found in the resorbing bone … earlier (Fig 9A) When the otosclerotic … was completely mineralized it showed …



7 (A) A portion of an osteoblast act vely engaged n eformation s shown n an act ve les on Unm neral zed agen f bers are seen nearby the osteoblast contain ng arged ER and swollen m tochondria Decalc f ed (B) A power v ew of os eoblast bone junct on shows re culin-l ke precollagen (*arrow*) and collagen f brils (*Coll*) Decalcif ed (C) An elec ron m crograph dep ct an osteoblast actively secret ng collagen f bers (*Coll*) and p eos eocyte (*pOc*) that s be ng walled off and an osteocyte (*Oc*) n partly mineral zed lacuna Decalcif ed

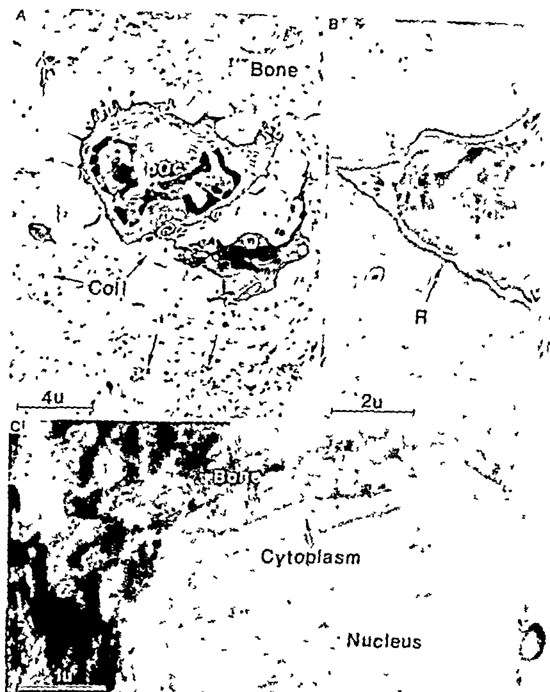
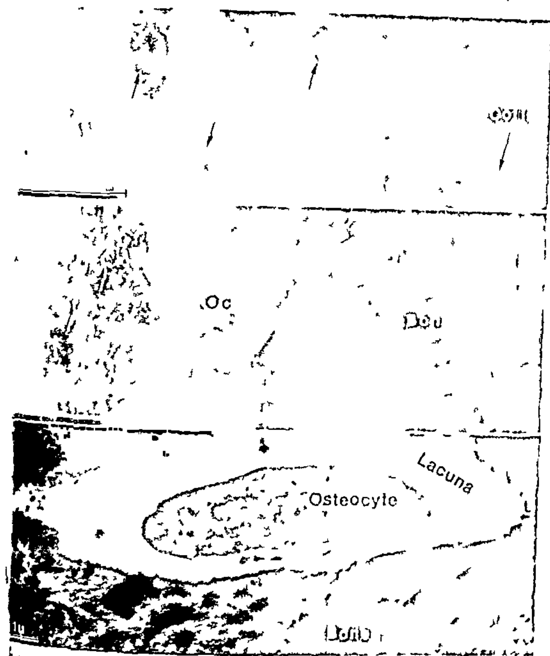


Fig. 8. (A) Preosteocytes (*pOc*) are seen in newly formed lacunae that are partly mineralized. Arrows point to nucleation centers of early mineralization. *Coll*: unmineralized collagen. Decalcified. (B) An osteocyte (*Oc*) is occupying lacuna with a distinct Rouget-Newman zone (*R*). Bone is well mineralized. Decalcified. (C) An os[illegible] nucleus, scanty cytoplasm and dark gran[illegible] occupies a well-mineralized lacuna in [illegible]. Decalcified.

mely irregular patterns of crystal deposition due to the irregular collagen fiber arrangement which was in contrast to the normal bone where the fibers were organized in lamellar fashion (Fig. 9B and C).

DISCUSSION

There have been numerous hyp[illegible] concerning the pathogenesis of the oto[illegible] is generally recognized that the devel[illegible] and progression of the otosclerotic lesion [illegible]



(A) Calcif cat on centers (calcospherites) are ind by arrows n an active otosclerot c les on Bone ral depos t appears to be closely related to the phous substance (*arrows*) *Coll* unm neralized col L Undecalc fed (B) A well m neral zed otosclerot c lesion shows irreg lar p ttern f cryst llites wh ch often f rm whirls (rr ws n ste cyte (*O*) appe rs degener ated Un lec lc fe l (C) A ste cyte in n en f p rt f stapes with well rg / e l ne U lec lcifie l

in several stages (1) destruction of the en ndral bone mediated by osteocytes and oclasts (2) formation of immature baso ic bone and (3) repetition of the remodel process of resorption and new bone forma tion which eventu lly l ecomes highly minera lized and cidophilic h ving n mos ic like ppe r nce (Schuknecl 1974) This concept of different stages is also supported by en zyme histochemic l studies (All ern z &

Covell 1961, Alberti & Tarkannen 1963) On the other hand Kelemen & Linthicum (1969) observed that every otosclerotic focus contained spongiotic and sclerotic portions and, therefore these two portions did not represent young and old lesions The present study did not cover the entire spectrum of the otosclerosis but it was concerned mainly with the pathology involved in an active spongiotic lesion

It is generally accepted that the otosclerosis develops in predilected areas of the temporal bones and stapes For frequent occurrences in certain families the genetic factors in the development of this disease have been implicated It is well recognized that genetic disorders of connective tissue can also be manifested in hypophosphatasia in which the bone lesion is caused by the inability of the osteoblasts to elaborate calcifiable matrix due to alkaline phosphatase deficiency and in osteogenesis imperfecta in which collagen is anomalous in its amino acid sequence (McLean & Urist 1968) Holdsworth et al (1973) suggested that the defective regulation of glycolysis and the resultant increase in aerobic metabolism by an aberrant phosphofructokinase (PFK) enzyme are the underlying causes of the otosclerosis Recent electron microscopic studies also reported the presence of abnormal collagen fibers and bone minerals in otosclerotic bones (Reydon & Smith 1968 Meurman et al 1968) These abnormalities were inferred to be the result of malfunctioning bone cells which produced abnormal ground substances

In our study the newly formed collagen fibers that walled off new preosteocytes were not well mineralized Whether the failure of mineralization was due to the peculiarity of the matrix formed by bone cells (Arslan & Ricci 1963) or due to the failure of bone cells to mobilize bone minerals could not be determined on the basis of a morphological study alone However it appeared that the newly formed bone lacked dense ground substance in which bone minerals were ordinarily depos-ited There was no gross abnorm[...] llagen fibers per se in the lesi[...] rangement lacked organization[...] We could not be certain whether [...] in the osteocytes in newly formed [...] resent pathological changes They [...] represent different physiological s[...]

Fetter & Capen (1971) reprod[...] tive changes of osteoblasts and [...] normal pig turbinate by inocula[...] rials obtained from nasal turb[...] spontaneously developed atrop[...] Osteocytes in the experimental [...] showed marked swelling of [...] which was not seen in normal c[...] mens The swelling of mit[...] osteoblasts in active otosclerot[...] served in this report might be th[...] reason for the malfunctioning of [...] since the mitochondria houses [...] zymes needed for energy metabo[...] transport We considered the po[...] the mitochondrial swelling was [...] artifact However in view of w[...] cell organelles including mitochon[...] bone cells in the same section w[...] concluded that the mitochondrial [...] antemortem Further cytochemica[...] be required to determine whethe[...] mitochondria in these osteoblasts [...] lesion are malfunctioning

Chevance et al (1970) recentl[...] that osteocytic osteolysis was the p[...] resorbing mechanism in the in[...] lesion They further suggeste[...] hydrolases released from lysosom[...] the osteocyte caused demineraliz[...] bone These authors were able to [...] seepage of the acid phosphatase [...] advancing front of the otosclerot[...] agreed with their findings that t[...] evidence of degranulated lysosom[...] tured cell organelles in the advan[...] appeared that enzymatic diges[...] lacunae initiated by osteocyte [...] hydrolases as suggested by Che[...] was the main mechan sm involved

bone resorption in otosclerosis although osteoclasts were found in active lesions neralized microfoci, described by earlier igators using decalcified tissue (Smith & n 1968, Chevance et al, 1970), were und in the present study utilizing unde- ed otosclerotic stapes These microfoci red to be related to the resorbing canali which chemical mediators such as acid lases could be easily transported

mechanism by which degranulation of mes is accomplished in otosclerosis is own It has been demonstrated that the yroid hormone and vitamin D activate or e lysosomal enzymes in bone cells Be- these systemic factors, it has been shown cal factors can, in part, regulate the bone tion and bone formation (McLean & 1968) Conceivably, the local factor reg- g bone resorption and bone formation, are under genetic control, are malfunc g in otosclerosis It is also conceivable he degeneration of capillaries in the mar- pace, observed in the present study, in- l otosclerosis, as it is known that degen n of the spiral vessels was the underlying anism of genetic cochlear degeneration in aker 1 mice (Kikuchi & Hilding, 1967)

ny investigators using light microscopy already reported the presence of clasts in an active otosclerotic lesion ner et al, 1960, Nager, 1969, Schuknecht, However, electron microscopic studies l to show osteoclasts in the surgically re d otosclerotic stapes (Reydon & Smith, Chevance et al, 1970, Lim, 1970, au 1971) On the basis of their failure to osteoclasts in the surgical specimens, ance et al (1970) felt that osteoclasts had a secondary function in resorption of bone tosclerosis Nevertheless, Gonzales & wksy (1961) confirmed the bone resorb- nction of the osteoclasts by showing bone- ite crystals (calcium phosphate) seen in the s of cytoplasmic processes and invaginated oles of the osteoclast The present find revealing that osteoclasts were actively engaged in the removal of the bony matrix in certain active otosclerotic lesions, agreed with earlier light microscopic reports (Henner et al, 1960, Nager, 1969) Therefore, it could be con cluded that both osteoclastic and osteolytic osteolysis might occur concurrently in certain active lesions It was also apparent in the pres- ent study that macrophages were actively en gaged in phagocytosis of dead cells and de stroyed bones Perhaps osteoclasts could help to dissolve bone minerals and collagen, but the waste products could be then taken up by the macrophages

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ZUSAMMENFASSUNG

Insgesamt zwe t n entkalkte und entkalkte oto- sklerotisch S m aktiven otosklerotischen Herden wurden ermikroskopisch untersucht In allen L nt u r rben konnten Zeichen von osteolytischer nachgewiesen werden Osteo klasten hing r en nur in vier Steigbugeln beobachtet D n kende Front der spongiotischen Laesionen ers t n folge der Demineralisierung der Canaliculi und t n wie von Motten zerfressen Der Demineral s erun n ess scheint durch eine De granulierung d r l s some durch degenenerende Osteozyten eingele t zu werden Wahrend die Knochen resorption fort chre tet wird durftig mineralisierter neuer Knochen dur h Osteoblasten die eine Schwellung ihrer Mitochondrien aufweisen angebaut Die mögliche Bedeutung d eser Schwellung der Mitochondrien fur die Pathologie der Otosklerose wird diskutiert

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D J Lim M D
Dept of Otolaryngology
The Ohio State University College of Medicine
456 Clinic Dr
Columbus Ohio 43210 USA

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INCORPORATION OF RADIOACTIVE CALCIUM INTO OTOLITHIC MEMBRANES AND MIDDLE EAR OSSICLES OF THE GERBIL

R E Preston, L -G Johnsson, J H Hill and J Schacht

From the Kresge Hearing Research Institute and the Department of Otolaryngology University of Michigan Medical School Ann Arbor Mich USA

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ract $^{45}CaCl_2$ was injected into gerbils in single or ple doses and the resulting radioactivity in serum onal $CaCO_3$ bone samples and selected labyrin epithelium was determined by liquid scintillation trometry Incorporation into both utricular and saccu toconia occurred at the rate of 0 06–0 07 nmole Ca^{++} day corresponding to a fractional rate of uptake of r The retention of radioactivity had a half-life of oximately 11 days The rate of incorporation of um for the middle ear ossicles was 5–7 times that for onia and was similar to that for otic capsule and tal bone The level of $^{45}Ca^{++}$ was higher in the ented regions of the utricular membranous wall than e non-pigmented areas of the utricular and ampullary and in the stria vascularis

otoconia in man and other mammals con- of crystalline calcium carbonate in the n of calcite (Carlström, 1963, Carlstrom & ström, 1955) unlike the mineral component ones and teeth which is principally calcium sphate as the complex salt carbonate- ite Ross & Peacor (1975) have recently n a detailed description of the otoconia in rat Contrary to popular belief the otoconia not, perhaps with the exception of rela- ly few crystals in the innermost layer, edded, in the true sense of the word, in the thic membrane (Johnsson & Hawkins,) They form a separate crystalline layer and are loosely held together by an organic substance which has not yet been identified

It is not known whether there is loss and renewal of otoconia in adult mammals In both fixed and unfixed material the otoconia can very easily fall off the membrane Schuknecht has suggested that in man utricular otoconia perhaps may become dislodged into the pos- terior ampulla and cause a form of disequi librium that he has termed "cupulolithiasis" (Schuknecht 1962 1969 Schuknecht & Ruby, 1973)

We have found (Johnsson, 1971, Johnsson & Hawkins, 1972) in the saccule from the cat both in vivo and post mortem a substantial number of loose otoconia below the otolithic membrane, accumulated along the inferior wall of the saccule It is quite possible that these crystals represent otoconia dislodged from the otolithic membrane Reduction in size and number of otoconia in the temporal bones from several aged human patients was also reported

There are thus indications that loss of otoconia, and perhaps a decreased production of them with aging, may occur in man and other mammals Yet there is no information in the literature concerning regrowth of otoconia in adult mammals There is a continuous growth of many fish otoliths (Pannella, 1971)

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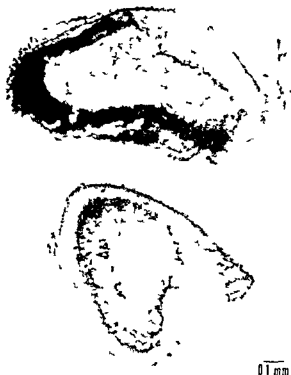


Fig 1 Macula sacculi (top) and utriculi (bottom) from gerbil dissected out from the vestibulum Each neuroepithelium is covered by the otolithic membrane with its otoconia Left ear OsO_4

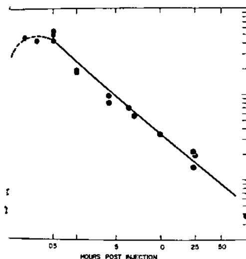
Dohlman (1971) has shown regeneration of the cupula in the pigeon after surgical ablation and Werner (1940) described regeneration of the membranous layer of the saccular superstructure in guinea pigs approximately three *months after the original* membranes had been dislodged by exposing the animals to strong centrifugal force Werner s drawing (1940, p 139) of the macula sacculi covered by the new otolithic membrane shows, however, no regeneration of otoconia Belanger (1960) showed some incorporation of calcium into otoconia of very young rats and also found that isolated and fixed otoconia were able to exchange ^{45}Ca in vitro with a solution in which they were immersed Recently Veenhof (1969), using autoradiography, was unable to *demonstrate ^{45}Ca incorporation into the* otoconia of adult mice

The present study was designed to investigate the possible growth or neogenesis of otoconia by measuring the uptake of ⁴⁵[illegible] gerbil was selected because relativel[illegible] doses of tracer could be given in rel[illegible] body weight, thus achieving a high [illegible] radioactivity in the serum This is ne[illegible] because most of the labelled calcium is taken up by bone An attempt was als[illegible] to study the incorporation of calci[illegible] pigmented and non pigmented portion[illegible] membranous wall of the utricle The te[illegible] of tissue dissection and liquid scin[illegible] spectrometry represents the most s[illegible] quantitative method for the measure[illegible] ^{45}Ca uptake

METHODS

Sexually mature male and female [illegible] *(Meriones unguiculatus)* weighing [illegible] were used in these studies Approxima[illegible] ml saline containing 0 4 or 4 μCi [illegible] *body weight was injected* intrapenit[illegible] For serum analyses the animal was anesthetized with ether and about 0.[illegible] blood was obtained from the orbit T[illegible] was allowed to retract overnight in t[illegible] and residual erythrocytes in the [illegible] serum were removed by centrifugatio[illegible] quots were analyzed for calcium by t[illegible] solphthalein complexone method (Gi[illegible] 1967) and for ^{45}Ca by liquid scint[illegible] spectrometry

Tissue samples were obtained by m[illegible] section techniques described els[illegible] (Hawkins & Johnsson 1975) The m[illegible] with their otolithic membranes are sh[illegible] Fig 1 Initial fixation of the inner ear [illegible] was accomplished by gentle perfusion [illegible] buffered OsO_4 into the widely opene[illegible] tibule Small samples of bone (ca 50 μ[illegible] the otic capsule near the *superior* ampu[illegible] from the midshaft of the femur were pic[illegible] broken off and carefully cleaned of m[illegible] *and periosteal soft tissue* under the diss[illegible] microscope The temporal bones and sl[illegible] samples were immersed in the OsO_4 s[illegible] for 24 hours and then partially dehydra[illegible]



Specific activity of serum ^{45}Ca in adult gerbils of sexes after single injections (4 μCi $^{45}CaCl_2$ per gram weight intraperitoneally) plotted as a power function beginning 30 min after injection Serum calcium concentration is assumed to be 2 2 mM for all animals

ethanol Samples of this solution did no measureable contaminating radioactivity All specimens were transferred to alcohol, which was changed several times during dissection The fixation time was longer than usual because this in our experience makes the otoconia more adherent to the membrane and surrounding crystals Nevertheless, several otoconia are often lost from the surface of the crystalline layer, particularly in the case of the saccule With care it is possible to dissect out samples uncontaminated by bone dust Several otoconial samples obtained from experimental animals, including gerbil, have been examined by powder X ray diffraction They have shown no traces of bone in the form of apatite

Weights of otolithic membranes and bone samples were obtained after overnight drying on pre weighed foil squares The samples were then dissolved in HCl and the radioactivity measured

Small pieces of utricular and ampullary membranous wall and stria vascularis from the lower basal turn were sampled as described below The tissue samples were digested in hot 0 25 M NaOH and neutralized prior to scintillation counting

RESULTS

Serum calcium

The concentration of calcium in the serum was determined from 4 animals to be 2 21 mM±0 03 All further calculations are based on this value After a single injection of 4 μCi

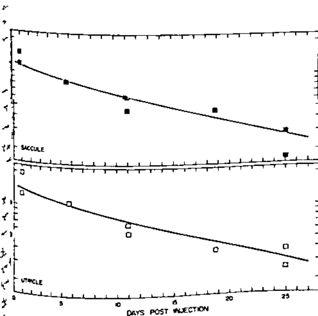


Fig 3 Total cpm ^{45}Ca for the paired otolithic membranes from each experimental animal plotted as exponential functions The lines were fitted to the data points by the least squares method Adult gerbils males and females $^{45}CaCl_2$ 4 μCi/g body weight intraperitoneally

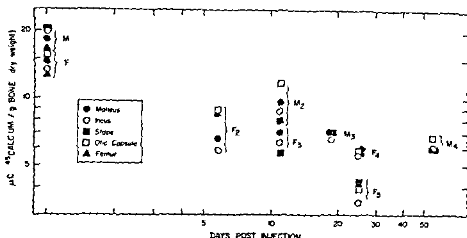


Fig 4 Selected determinations of ^{45}Ca in bone samples from gerbils The counts were obtained from whole middle ear ossicles and samples of comparable size taken from the bony ampullae and from femoral cortical bone All samples had been exposed to OsO and dried M males F females $^{45}CaCl_2$ 4 µC intraperitoneally

$^{45}CaCl_2$/g body weight the highest level of radioactivity in the serum is reached between 20–40 min (Fig 2) There were no differences in the specific radioactivity of serum calcium between males and females

Calcium turnover in otoconia and bone

Fig 3 shows the retention of ^{45}Ca in saccular and utricular otolithic membranes in eight animals which received single injections of isotope 4 µCi/g body weight Total counts are plotted rather than activity on a weight basis since weight determinations proved to be imprecise The lines were fitted to the data points using the least squares method and best fit a straight line on a semilogarithmic plot The half life of calcium in utricular and saccular otoconia is approximately 11 3 days Incorporation in an adult gerbil was compared to that in a six week-old weanling after ten daily injections of $^{45}CaCl_2$ Based on the specific radioactivity of calcium in the serum no difference was found in the uptake of calcium into otoconia in this experiment

Incorporation and retention of ^{45}Ca in middle ear ossicles was measured in the same animals and was similar to that in neighboring otic capsule and typical skeletal bone (Fig 4) Higher values were found for all bones in 4 male gerbils (M_1–M_4) compared to five female gerbils (F_1–F_5) whereas no such was found for the otoconia In a experiment in which 40 daily doses $^{45}CaCl_2$/g body weight were given tion again was similar for the th and the other bone samples

Uptake of calcium into the membranous walls

Uptake of ^{45}Ca into different p membranous walls was measure single or multiple injections of ^{45}C vestibular system the pigmented m wall and portions of the wall wh completely non pigmented were These regions are clearly outlined recognized under the dissecting m The non pigmented samples were the anterior end of the utricle close tical recess and from the dome of t tal or superior ampulla Samples of laris were also taken to serve as contaminating radioactivity in bl cause the marginal cells of stria m dark cells associated with pigmen the vestibular labyrinth Strial sa dissected loose from the spiral liga lower basal turn Paired or triplet pigmented and non pigmented stria vascularis were cut out from t

e I ^{45}Ca uptake (cpm) into labyrinthine elia

als received 4 μCi $^{45}CaCl_2$/g body weight daily for the er of days indicated and were sacrificed one day he last injection except *, sacrificed six days after jection

f ent	Pigmented utricular wall	Non pigmented utricular wall	Ampullary wall	Stria vascularis
	35	–	15	–
	42	–	–	16
	23	–	8	16
	63	29	–	–
	254	–	106	–

small iris scissors The areas of the pieces estimated to be within 10% of each other he highest incorporation was found in the nented samples (Table I) Those from stria ularis and non pigmented areas of the cular and ampullary walls contained asurable but significantly lower radioac y

trol experiment

ontrol experiment was performed to in that ^{45}Ca in samples of otoconia and rinthine epithelium was not due to con ination by blood or body fluids An animal given 2 5 μCi $^{45}CaCl_2$/g body weight and ed after 30 min when ^{45}Ca in serum was its highest level (Fig 2) Radioactivity in serum was 1500 cpm/μl, but otolithic mbranes, *cupula and utricular epithelium* no activity One of two samples of stria culans contained 17 cpm ^{45}Ca

DISCUSSION

lcium turnover in otoconia

contrast to the well-established morphology l composition of otoconia little is known ut their origin and their turnover In poration of calcium into these structures been shown *in young animals* (3 to lay-old rats Belanger 1960, fetal to 2 y-old mice Veenhof 1969) The present study clearly demonstrates incorporation of Ca^{++} into otoconia of adult as well as weanling gerbils The rate of incorporation can be calculated from the radioactivity in the otolithic membrane one day after injection (Fig 3) and the integral of the specific radioactivity in the serum over the same period (Fig 2) This rate is 0 06 and 0 07 nmole calcium per day for the otoconia of the saccular and utricular membrane, respectively The retention curves for calcium in the two types of membranes are almost identical (Fig 3) and indicate a half life of otoconial calcium of approximately 11 days

In order to compare the metabolic activity of the otoconia to that of the surrounding bone the weight of otoconial $CaCO_3$ must be determined Because of the small mass, measurements on a large pool of otolithic membranes would be required On the basis of eight determinations each we estimated the weight of otoconial $CaCO_3$ to be 9 μg per pair of saccular *and 17 μg per pair of utricular* membranes This leads to a calculated fractional calcium uptake of 0 08% and 0 12% per day for saccular and utricular otoconia respectively Thus calcium incorporation into these structures is 5–7 times slower than into the surrounding bone (see below)

Incorporation into bone

It is evident that the uptake and retention characteristics are similar in the middle ear *ossicles the neighboring otic capsule bone and* the femur (Fig 4) The dry weight of the ossicles of the gerbil were determined to be malleus 1 107 μg±55 S D (n=18) incus 657 μg±53 *(18) stapes 126±18 (10)* Assuming a calcium concentration of 22% in bones of all sources (Irving 1973, Vignen et al 1973) the fractional incorporation rate of Ca^{++} into the various bone specimens was calculated (Figs 2 and 4) to be 0 70% for a male (M_1) and 0 47% for a female (F_1), one day after a single injection of $^{45}CaCl_2$ The higher incorporation of calcium into bone of male gerbils may indicate a sex difference, but it should be

that the female gerbils were obtained from a different supplier.

The essentially identical rate of calcium uptake for all three middle ear ossicles is in apparent contrast to a study by Vigneri et al (1973) In the weanling rat these authors found the stapes to be much more active metabolically than the other ossicles Differences in experimental conditions preclude direct comparison with the present results so that it remains unresolved whether this interesting metabolic variance is age or species dependent

Utricular "dark cells"

On morphological grounds Lim (1973) has suggested that the specialized, osmiophilic or electron-dense epithelium, termed "dark cells", overlying melanocyte-containing subepithelial tissue in the vestibular labyrinth is responsible for removal of calcium from the otolithic system Crystals dislodged from the otolithic membrane appear to lose their mineral content when attached to the dark cells. We have subsequently observed this relationship, in both light and electron microscopy, between otoconial crystals and the dark-cell epithelium in humans, as well as in guinea pigs and squirrel monkeys Localization of ^{45}Ca activity in pigmented areas and thus presumably in the dark cells can be observed in our experimental animals (Table I) Higher dose levels of ^{45}Ca, together with quantitative control of amounts of tissue removed from different animals, might permit the determination of the time at which maximum activity appears in the dark cells and thus test the implication that they are responsible for the removal of otoconial $CaCO_3$ from the endolymph It should be noted, however, that no dark cell epithelium has been described in the mammalian saccule The fate of the saccular otoconia and the possible involvement of the specialized epithelium of the endolymphatic sac in their removal remains unclear

In summary, this study has provided evidence for an active turnover of calcium in otoconia, and has shown that in the ad[illegible] bil calcium metabolism in middle ear o[illegible] is typical of bone in the skeletal syst[illegible] addition it has been demonstrated th[illegible] dark cell epithelium contains higher a[illegible] of ^{45}Ca than surrounding tissue sugges[illegible] active participation of this tissue [illegible] calcium metabolism of otoconia Th[illegible] siological interpretation of the uptal[illegible] retention data for otoconia must rema[illegible] at this point, however The observed [illegible] may reflect growth of existing c[illegible] neogenesis of otoconia, simple [illegible] Ca^{++} between endolymph and ot[illegible] $CaCO_3$, or a combination of these pro[illegible] Further investigations will be necess[illegible] clarify these questions

ACKNOWLEDGEMENT

Dr Roland Rouse of the University of Michiga[illegible] ment of Geology performed the crystallographic [illegible] James W Bruce and Vernon T Maulbetsch co[illegible] artistic and photographic assistance We thank P[illegible] Hawkins, jr for advice and critical reading of th[illegible] script

ZUSAMMENFASSUNG

Gerbils (*Meriones unguiculatus*) erhielten einma[illegible] mehrmalige Injektionen von $^{45}CaCl_2$ Die resul[illegible] *Radioaktivitat im Serum im* $CaCO_3$ der Otol[illegible] Knochen und Teilen der hautigen Wand des [illegible] wurde im Scintillationszahler gemessen Einbau [illegible] in die Otolithen der Macula sacculi und utricu[illegible] mit einer Geschwindigkeit von 0 06–0 07 µMolen [illegible] pro Tag entsprechend einer prozentualen Einba[illegible] 0 1% Die Retention der Radioaktivitat zeigte e[illegible] wertszeit von etwa 11 Tagen *Die Einbaurate von* [illegible] war 5–7 mal höher in den Gehörkn[illegible] *Otolithen und* [illegible] set Der Ge[illegible] gen Wand [illegible] menberten [illegible] Ampulla sow[illegible] vascularis

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R E Preston A B
Kresge Hearing Research Institute
University of Michigan Medical School
Ann Arbor Mich 48104 USA

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INTERMEDIUS NERVE INVOLVEMENT AND TESTING IN ACOUSTIC NEUROMAS

J Thomsen and K Zilstorff

From the ENT and Neurosurgical Departments University of Copenhagen Rigshospitalet Copenhagen Denmark

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Abstract The clinical findings in 125 patients with surgically confirmed acoustic neuromas are presented with special regard to the involvement of the intermedius nerve in the diagnosis In assessing the function of the intermedius nerve the examination of the nasolacrimal reflex and the sensation of taste on the anterior two-thirds of the tongue are used The methods of investigation are described in detail The material consisted of 20 medium sized and 105 large tumours no intracanalicular tumor was found Hearing loss was the initial symptom in 85% of the patients 10% had tinitus and 4% vertigo as the first symptom Apart from the VIII cranial nerve symptoms a defective nasolacrimal reflex was the most significant evidence of cerebellopontine angle pathology The test was positive in 65% of the medium sized tumours in the entire material 85% The figures are higher than the incidence of trigeminal nerve symptoms This in contrast to the reports of most authors The tests described are simple and quick to perform and it is emphasized that they should be applied to all patients with unilateral hearing loss of unknown origin

[Th]roughout the years a connexion between [th]e size of the acoustic neuroma and the morbidity and mortality at operation has been clearly demonstrated The larger the tumour, the greater the morbidity and mortality

However, the early diagnosis though much improved in the past 25 years, has by no means paralleled advances in the surgery of acoustic neuroma The diagnosis of these tumours at an early stage while they are still small, is important, since total removal is possible in most cases, with very low mortality

This work has been supported by a grant from the P Carl Petersen Foundation (Grant B 921)

and little or no damage to the most cases

The purpose of this paper i clinical findings in 125 patients confirmed acoustic tumours gard to the involvement of termedius in the diagnosis

The motor fibres supplying metic musculature form the part of the facial nerve, but in portion made up of visceral an ent and general visceral effere This minor part is called the in or the sensory root of the fa general visceral efferent fib lacrimal, the submaxillary an glands as well as the glands part of the oral cavity, and glionic parasympathetic (secr these structures The taste fi 2/3 of the tongue passes cent intermedius nerve Cutaneou sensory branch of the facial auricle and external auditor conducted centrally via the int In addition these regions are sensory auricular ramus of (Brodal, 1969) In the interna the intermedius nerve lies be nerve above and the nervus below, hence the name

Diagram of the course of the nasolacrimal reflex (heavy lines)

assessing the function of the nervus in-[illegible]dius we use the examination of the [n]acrimal reflex (NLR) and the taste on the [ante]rior 2/3 of the tongue

[Th]e nasolacrimal reflex consists in lacrima[tion f]ollowing chemical or mechanical stimula[tion o]f the nasal mucosa The trigeminal nerve [(oph]thalmic and maxillary branches) form the [affer]ent paths Its efferent paths proceed from [the] superior salivary nucleus along the in[term]edius nerve, through the geniculate gangl[ion,] the greater superficial nerve, through the [geni]culate ganglion, the greater superficial [petr]osal nerve, through the nerve of the [ptery]goid canal (vidian nerve) to the sphe[nop]alatine ganglion From here they pro[ceed] through the sphenopalatine nerves to the [max]illary nerve and via the zygomatic nerve to [the] lacrimal nerve and the lacrimal glands [(Fig] 1)

In 1900, Koster found that the secretomotor fibres to the lacrimal gland proceed along the above-mentioned course However, Wernoe was the first to describe the whole reflex in 1929 That the reflex path takes this course has been proved in operated tumour cases by Zilstorff in 1959 The taste sensation of the anterior two-thirds of the tongue is conveyed centrally through the gustatory fibres in the lingual nerve, the chorda tympani, the intermedius nerve to the nucleus tractus solitarius (Cushing, 1971)

METHODS OF INVESTIGATION

To elicit the nasolacrimal reflex the following technique is used One end of a piece of filter paper, 0 5×0 10 cm is inserted into the lower conjunctival fornix and folded twice at right angles over the ciliary border of the lower lid,

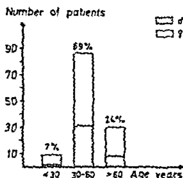


Fig 2 Age distribution of 125 patients with acoustic neuromas

at the inferior lacrimal punctio The paper is left in position while the examination takes place For quantitative stimulation of the nasal mucosa the Elsberg olfactometer (Elsberg 1935, Zilstorff, 1959) is used, with benzine as the trigeminus stimulant Benzine acts in most patients as a potent trigeminal stimulant and lacrimator After the filter paper has been placed in the fornix a stream of saturated benzine fumes, 500 ml/min , is blown through one nostril directed at the olfactory region, for 30 sec while the patient breathes through the mouth The paper is left in place for another 30 sec before it is removed When the paper has dried, the lacrimation can be measured directly in millimeters After an interval of at least 10 min the stimulation is repeated on the contralateral side It is important to maintain this time interval, as the function may be reduced on the contralateral side for some time, due to overflow through the choana

The lengths of the soaked tissue pieces on each side are compared measured in millimeters, a difference is considered significant if it exceeds 20% (Zilstorff 1959)

The sensation of taste is investigated by three different concentrations of the four basic qualities by the method of Bornstein (1940)

MATERIAL

The material consists of 125 patients with acoustic neuromas, operated in the department of neurosurgery, University [...] (Rigshospitalet) in the period 195[...] same physician (Z) has perform[...] otoneurological examination of all [...] tients An exact estimation of the tum[...] is difficult and the findings are often m[...] differently by otologists and neuros[...] However, we have followed the class[...] given by Pulec et al (1971) Small [...] (intracanalicular) are confined to [...] auditory canal Medium sized tumou[...] into the cerebellopontine angle with[...] out V nerve involvement but with n[...] brainstem or cerebellar symptom[...] tumours have indications of othe[...] nerve involvement cerebellar or [...] symptoms, and eventually increased [...] nial pressure

In the material, 20 medium-sized [...] large tumours were found The smal[...] were not represented

The age distribution is seen in Fi[...] majority are found in the 30-60 [...] group—these tumours are rare below [...] of age Women outnumbered men [...] one

In Fig 3, length of history is [...] tumour size Only 26% were examin[...] the first year and 38% had a histo[...] than 5 years There is a tendency to[...] history in the medium sized tumour[...] but no definite connection between [...] history and tumour size can be es[...]

In Fig 4 the patients' indications [...] symptom are shown In 85% hearin[...] the initial symptom 10% indicate[...] and 4% vertigo as the first sympto[...]

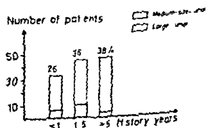


Fig 3 Length of history in relation to tumo[...] patients with acoustic neuromas

atients

HL = Hearing loss
T = Tinnitus
V = Vertigo
O = Other

10% 4% 1%

T V O Symptoms

rst subjective symptom in 125 patients with uromas

patient indicated reduced facial ty initially

tient in the entire material had normal Among the 20 medium-sized tumour 15 had only impaired hearing, while 5 cusis in the ear involved Among the mour patients considerably more had (65%), while 35% had impaired hear (Fig 6)

of the patients with medium-sized had abolished differential – caloric even after irrigation with 20° water n (Fig 5), while this occurred in 84% he patients with large tumours (Fig 6) tients had normal caloric function

incidence of central spontaneous us and central positional nystagmus is Figs 5 and 6 It should be noted that ngle patient with medium-sized tumour efective optokinetic nystagmus, while the patients with large tumours had and 8 show a comparison between the ms from the trigeminal and intermedius The trigeminal function is estimated facial sensation and quantitative sensitivity (*ad modum* Boberg-Ans. while the intermedius function is ed by the taste test and the nasolacri flex examination as described above It arly seen that in the medium sized s both taste and (especially) the NLR nsiderably more frequently involved he symptoms from the trigeminal nerve

(Fig 7) In the large tumours the pattern is the same (Fig 8), but the differences less pronounced It is evident, however, that loss of taste sensation and/or a defect NLR are found *in the vast majority of the cases (85%) (Fig 8)*

The motor function of the facial nerve and the sensibility of the external meatus have likewise been examined in the patients Among the 20 medium-sized tumour patients, one had impaired motor function and impaired sensibility We ascribe this to a previous zoster oticus in the involved ear 23% of the patients with large tumours had impaired motor function of the facial nerve, while only 6% had deminished sensibility in the posterior canal wall

The material includes 7 bilateral tumours, and while neuromas as a whole are rare below the age of 30 (Fig 2), in our material only 7%, we found that 4 out of 7 patients with bilateral neuromas are less than 30 years old All these 4 young patients had signs of Recklinghausen's disease, while the remaining 3 patients had no signs of this disease

In the material 6 patients had recurrence of a neuroma (5%)

DISCUSSION

As mentioned earlier, it is of importance for an improvement of the postoperative morbidity and mortality to detect acoustic neuromas as early as possible or, more correctly, to find them when they are as small as possible In

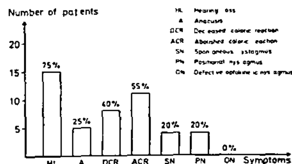


Fig 5 VIII nerve symptoms in 20 patients with medium sized acoustic neuromas

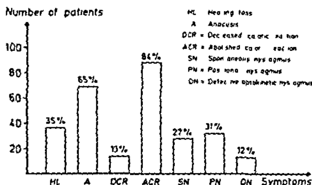


Fig 6 VIII nerve symptoms in 105 patients with large acoustic neuromas

our material none of the small, intracanalicular tumours were to be found, and only 20% of the tumours could be classified as medium sized This distribution corresponds fairly well to the reports of other authors, e g Ozsahinoglu & Harrison (1974) and Ellis & Wright (1974) Some authors have been able to report materials with a considerable preponderance of small and medium-sized tumours, e g Fisch & Wegmüller (1974), House & Graham (1973), Montgomery (1973)

Regarding length of history, age and sex distribution, this material does not diverge from the reports of others (Cushing, 1917, Johnsen & Kristensen, 1949, Lundborg, 1952, Rembold & Tonnis 1958, House, 1968, Fisch & Wegmuller, 1974, Ozsahinoglu & Harrison, 1974) With regard to the value of the examinations for the intermedius nerve involvement in the diagnosis of acoustic neuromas the opinions varies considerably Fisch & Wegmuller (1974) do not mention these examinations Sheehy (1968) states, that the examinations are routinely performed, but that they are of no differential diagnostic importance

In our material we find that apart from the eight cranial nerve symptoms a defective nasolacrimal reflex is the most significant evidence of cerebellopontine angle pathology Among the medium sized tumours the test was positive in 65%, in the entire material 85% These figures are considerably higher than the incidence of trigeminal nerve symptoms, evaluated by facial and corneal sensitivity

The trigeminal nerve involvement was among the medium sized tumours and among the large This stands in contras reports of most other authors (Cushing, Parker, 1928, Olsen & Horrax, 1944 E & Paterson, 1951, Lundborg 1952, Cl & Garvey, 1953, Pool & Pava 1957 (orne, 1969, Axelsson et al , 1973) Ho these authors have not used the nasol reflex examination, as described by Z (1959)

Among the medium sized tumours taste sensation of the anterior two-third tongue is also more frequent than the t nal symptoms The examinations of t is performed *ad modum* Bornstein as *quantitative examination* This method our experience, proved to be more p and easier to perform than electrogus (Krarup, 1959) in the clientèle from n gical departments

There are several methods testing t tion of the parasympathetic fibres in termedius nerve Schirmer's (1903) test was an oculo lacrimal test Bob (1955) described a variation of this te sessing decreased lacrimation, and t test used by Pulec & House (1964) (1971) has compared the Pulec & method with that of Zilstorff (1959) a that the quantitative measurement ad Zilstorff is more sensitive and reliable

Among the medium sized tumours w only one patient with reduced mot

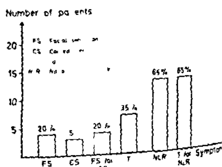
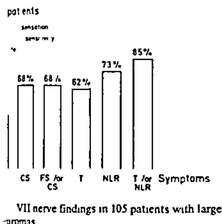


Fig 7 V and VII nerve findings in 20 pat medium sized acoustic neuromas



VII nerve findings in 105 patients with large
-uromas

and ascribed this to a previous zoster
olved ear Regarding the reason for
at the sensory and parasympathetic
e facial nerve more frequently suffers
ssure of the tumour we may only
t the motor fibres are more resistant to
than the small sensory fibres (Gasser
er 1929, Gasser, 1935, MacGladery
))

the reduced sensibility of the
wall of the external meatus as de-
, Hitselberger & House (1966) and
r & Feldmann (1973), we have been
find this symptom with any reasona-
ee of frequency This may be due to
iable innervation of these regions,
the vagus nerve, partly the in-
e nerve (Brodal, 1969)

reported in 1973 that a defective
etic nystagmus was a symptom with a
cy of 50% in large acoustic neuromas
e not been able to confirm this, since
e only found a defective optokinetic
is in 12% of the large tumours
cases adherence to or impression in
in stem was found, but clinically this
nly be confirmed in 12% (by presence
act signs) It is however, not the same
who have long tract signs and defec-
netic nystagmus, since only 2 pa-
both these symptoms The brain
probably affected a considerable time
before significant impairment of the opto-
kinetic nystagmus occurs

The early diagnosis of acoustic neuromas is of decisive importance for the course of the treatment We believe that performance of NLR and taste examinations should be a routine part of the test battery used in the diagnosis of acoustic neuromas They are simple and quick to perform, they do not demand any expensive instruments, and any otologist should perform them when dealing with a unilateral hearing loss of unknown origin

ZUSAMMENFASSUNG

Die klinischen Befunde bei 125 Patienten mit operativ bestatigtem Akustikusneurinom werden vorgelegt und zwar mit besonderer Berucksichtigung der Einwirkung des Nervus intermedius Um die Funktion des Nervus intermedius zu bewerden werden der Nasolacrimalreflex und die Geschmacksempfindlichkeit der vorderen zwei Drittel zur Zunge untersucht Die Untersuchungsmethoden werden eingehend beschrieben Das Krankengut besteht aus 125 Tumoren 20 mittlerer Grosse und 105 grossen intracanaliculare Tumoren wurden nicht gefunden Bei 85% der Patienten war das erste Symptom ein Hörverlust Abgesehen von den Symptomen des 8 Hirnnervs war ein defekter Nasolacrimalreflex der bedeutendste Beweis einer Kleinhirnbruckenwinkelpathologie Der Test war bei 85% der Falle des gesamten Materials positiv Diese Zahlen sind hoher als das Vorkommen trigeminaler Nervensymptome Dies widerspricht den meisten bisherigen Berichten

Die beschriebenen Teste sind einfach und leicht anwendbar und es wird sehr empfohlen sie bei sämtlichen Patienten mit einseitigem Horverlust unbekannter Ursache durchzuführen Dadurch wurde die Anzahl grosser Tumoren (80% in unserem Krankengut) wesentlich reduziert

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J Thomsen M D
University ENT Department
Rigshospitalet
Blegdamsvej 9
DK-2100 Copenhagen
Denmark

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BIOLOGICAL CONSIDERATIONS FOR THE USE OF HOMOGRAFT TYMPANIC MEMBRANES AND OSSICLES

W Kuijpers and P van den Broek

om the ENT Department St Radboud Hospital University of Nijmegen Nijmegen The Netherlands

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This report deals with some biological aspects of tation and preservation of tympanic membrane ular grafts The structure and behaviour of membrane grafts depends largely on the reaction servatives with the tissue proteins Due to these s no observable change in the microscopical of the membranes and ossicles was found up to 3 er alcohol and formaldehyde preservation Pre in cialit showed a gradual disappearance of nuclei creas ng disintegration of the fibrous structures ive of the mode of preservation the fibrous tissue l to persist virtually unaffected for long periods nocellular reaction found around the grafts pre n formaldehyde after heterolopie transplantation that tissue antigens are not completely destroyed on to transplantation in the middle ear is only ound fresh heterologous grafts and around the mologous grafts after previous sensitisation As in rats ossicles preserved in alcohol or cialit from revision operations in humans showed emodelling Moreover in some cases these os nd especially the cartilaginous grafts showed areas erosion

e of homograft tympanic membrane and s in tympanoplasty has proved its value cent years Although there might still be sion as to whether superior results can ieved with autograft fascia or ossicles, authors who have reported their ex ce with homografts agree that there are iatable advantages in their use The re- obtained are mostly founded on clinical ence, while basic knowledge is still grow- confirm their value and to adjust the omings of the techniques used

history of the use of homograft tympanic ranes is comparatively short Chalat (1964) is generally given the credit to be the first to have used vital homograft tympanic membranes for myringoplasty Donor tym panic membranes were removed within 4 hours after death and kept in Ringer's solution for direct use In 3 patients the grafts were placed over the de epithelialised tympanic membrane of the acceptor Two of the grafts failed to be incorporated, the third reperforated later

Only 2 years later, Marquet (*1966*) reported on the use of 17 preserved cadaver tympanic membranes and his experience was much more encouraging The grafts were obtained within 12 hours of death, the epithelium was removed and the graft was stored in cialit (1 5000 solu tion) for about 4 weeks In this first series of 17 patients, complete closure of the perforation was obtained in 15 In 1968 Marquet reported on 283 myringoplasties with a primary take rate of 81%, and another 14% take rate after revi sion Improvement of knowledge and techni que has raised the primary take rate to about 85%, which equals the results with temporalis fascia MacKinnon (1972) describes the results of 112 tympanoplasties performed with homograft tympanic membranes preserved in 1 5000 solution of thiomersalate The primary success rate was 82% In a few cases he observed changes which could indicate inci- pient rejection Histological sections of parts of the grafted tympanic membrane removed at a



2a



2b



2c

Fig 2 Micrograph of tympanic membranes preserved for 3 years in formaldehyde (*a*) (pars flaccida) alcohol (*b*) (pars flaccida) and cialit (c) (pars tensa) Note the disintegration of the fibrous tissue after cialit preservation Only a few faint remnants of the nuclei can be seen in the fibrocartilaginous part of the annulus H E ×300

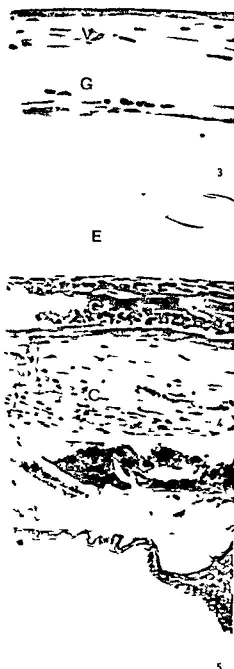
studying the effect of the preservati various tissue components were pr the same way After preservation and formaldehyde the specimens mediately decalcified in EDTA vation in cialit the grafts were firs formaldehyde 4 % or alcohol for 24 subsequently decalcified

RESULTS

Effects of Preservation on Structure of Graft

Cialit, ethanol, and formaldehyde a servatives which kill micro-organism der the autolytic enzymes inactive to cialit, alcohol and formaldehyde molecular structure of the tissue pr foundly With alcohol being a no fixative in the sense that none o stituents joins itself to the proteins teins are profoundly changed with coagulation Formaldehyde is an (reacts with protein) non coagulan which hardens a protein gel without the water from the protein Due to perties preservation in alcohol re shrinkage and hardening of the membrane Formaldehyde also has a effect but shows less shrinkage Pres cialit leaves the membrane very so able, and sometimes results in a sp loosening of the epithelial layer fr brous layer within a few weeks No of decalcification was found in th after 3 years storage in the neutra maldehyde solution No essential d can be observed between the micr structure of ossicles and tympanic m preserved in alcohol or formaldehyd hematoxylin eosin stain Twenty of mens were preserved for more tha without any significant change

Preservation in cialit during the sa shows a loss of nearly all nuclear sub slight disintegration of the fibrous stru an important loss of stainability with



Tympanic membrane 18 months after myringo- with a cialit preserved homograft The avital graft owing circular and radial fibres, is still present Vital formed fibrous tissue (*V*) external meatus (*E*) H E

cal and histochemical staining methods (Figs 1 and 2)

Effect of Preservation on Behaviour of Graft

Orthotopic transplantation

During the first week the tympanic membrane–malleus graft became surrounded by inflammatory cells, mesenchymal cells and capillaries The graft was covered within 2 weeks by many layers of squamous epithelium on the outer side and 2 to 3 layers of mucosal cells on the medial surface Thereafter the newly formed tympanic membrane gradually grows thinner These phenomena are very similar to those described previously when other tissues were used for myringoplasty (Reijnen & Kuijpers, 1971)

After 3 months the new membrane consists of a squamous epithelium consisting of 2 to 3 layers The middle layer shows the fibrous tissue of the graft on both sides covered by newly formed fibrous tissue of varying density with small capillaries On the medial side the membrane is covered by a thin mucosal layer Similar observations were made 18 months after transplantation In most cases the middle-layer of the graft appeared to be virtually intact, showing the characteristic fibre distribution with circular and radial fibres (Fig 3) After 18 months this characteristic distribution could not be observed in the newly formed fibrous tissue The persistence of the donor fibrous tissue after longer survival times agrees very well with the observations made in humans (Marquet et al , 1973)

Fig 4 Tympanic membrane 10 months after myringoplasty with a formaldehyde preserved graft The graft (*G*) is situated between a thin vital fibrous layer underneath the epithelium and a thick layer of loose connective tissue (*C*) containing round cells on the middle ear side The presence of this latter tissue was observed only in those ears in which the paraffin support was left in position External meatus (*E*) Heidenhain ×300

Fig 5 Micrograph of a tympanic membrane (with annulus) 6 months after myringoplasty with an alcohol preserved graft The new membrane mainly consists of loose connective tissue Note the foldings in the graft possibly due to bad positioning Heidenhain ×50

As in earlier observations, made with preserved incus grafts (van den Broek & Kuypers, 1974) the malleus attached to the tympanic membrane grafts and preserved in cialit or alcohol showed newly formed bone in some cases

In the animals in which the paraffin support was left in the middle ear, the tympanic membrane showed a persistent infiltration of inflammatory cells throughout the observation period (Fig 4)

The final thickness of the new membrane appeared to be highly dependent on the size of the graft Fig 5 shows a specimen in which the thickness of the membrane seems to be determined by the foldings in the graft No essential difference has been observed between the behaviour of the tympanic membrane grafts preserved in alcohol, formaldehyde or cialit

Heterotopic transplantation

The course of the reaction after heterotopical *transplantation of preserved tympanic membrane–malleus grafts* is very similar to that observed with preserved incudes (van den Broek & Kuypers, 1974) After preservation in *alcohol or cialit, new bone formation was* observed in most cases in the malleus, but not after preservation in formaldehyde Apart from this, formaldehyde preserved grafts appeared *to be surrounded by mononuclear cells* throughout the observation period, in contrast to those preserved in cialit or alcohol (Fig 6) A most remarkable finding was the extremely *high resistance of the fibro-cartilaginous part of* the annulus and the fibrous content of the grafted membrane to resorptive activity of the host (Fig 7) Even after 18 months the fibrous *tissue appeared to be nearly unaffected, ir*respective of the mode of preservation, while large areas of the malleus bone were resorbed

Fresh incus grafts after sensitisation

In contrast to the observations made on a large series of orthotopically transplanted fresh homologous incudes, which failed to show clear phenomena suggestive of a tr[...] tion reaction, resorption of *fresh in*[...] was observed in 3 out of 11 incudes [...] vious sensitisation This reaction wa[...] terised by the presence of a large n[...] mononuclear cells around the graft a[...] tive activity, with or without the pr[...] giant cells (Fig 8) No preserved incu[...] tested in this series

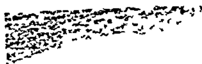


Fig 6 Micrograph of a tympanic membrane [...] formaldehyde 40 weeks after transplantation [...] *muscle The graft is surrounded by monon*[...] H E ×300

Fig 7 Fibrocartilage of the annular part of [...] membrane preserved in cialit 18 months after [...] tion into the leg muscle There is no react[...] graft and the structure is virtually unaffected



Fig 8 Fresh homologous incus graft 4 weeks after orthotopical transplantation in the middle ear of a previously sensitised animal The long process of the incus revealing a large area of bone resorption is surrounded and invaded by mononuclear cells Note the large giant cell H E ×90



ologous grafts

ses in which fresh heterologous incudes ransplanted orthotopically or heterotop a persistent massive reaction of nuclear cells around the grafts was pres uring the observation period up to 12 ıs Massive resorption of the transplanted es was a normal finding at both sites 9 and 10)

an specimens

ular grafts obtained from revision opera showed a great variety in histological ture Nearly all homologous ossicular s preserved in cialit showed areas of new formation In these ears which underwent ısion operation because of cholesteatoma urulent otitis large areas of the grafted les sometimes appeared to be resorbed or iced by fibrous tissue

omologous preserved ossicles obtained non inflamed ears which underwent revi because of dysfunction of the recon cted ossicular chain in many cases re ed new formed bone to a varying extent In w cases the graft was entirely dead A most arkable finding was that even in these non



Fig 9 Fresh heterologous incus graft (rabbit) 4 weeks after transplantation into the rat middle ear The graft shows large areas of resorption and is encapsulated in fibrous tissue with many mononuclear cells Stapes (S) H E ×90



Fig 10 Fresh heterologous incus graft (rabbit) 5 months after transplantation into the rat leg muscle Extens ve monocellular reaction and large areas of resorption H E ×90

inflamed cases some of the homografts sh clear areas of bone erosion (Fig 11)

In one case a large part of the graf replaced by fibrous tissue Apart fro bone erosion was also observed in one graft (mainly consisting of dead bone 6 after repositioning) Comparable res activity was observed in homologous ca grafts (preserved in alcohol) used for an ossicular reconstruction (Fig 12) and re because of poor functional results In al cases signs of inflammatory were v absent

DISCUSSION

The results of the experiments presen e study deal with several aspects of the homografts The first aspect which h dealt with is the influence of several pr tion modalities on the graft The resu sented show an important difference b the effect of preservation in alcoh formaldehyde versus preservation in c the physical properties and the micros structure of the tympanic membrane effects are due mainly to the differe these preservatives react with the tiss pounds of the graft especially the prot



F g 11 Incus graft in cialit removed f t ent because of dy months after transp Note small areas o orption (*arrow*) N nflammat on H E



Fig 12 Part of a cartilage graft preserved in alcohol, removed from the middle ear of a patient because of dysfunction after 18 months The cartilage is invaded and partially replaced by fibrous tissue H E, ×90

The resulting "fixation" involves ne histological structure of tympanic rane and ossicles after preservation in l or formaldehyde does not change with Even after 3 years the structure was nged

ause cialit does not interfere to any large with the solubility of tissue compounds, ation in an aqueous solution involves a al loss of many soluble substances This arent from a general loss of stainability, a earance of the nuclear substance of the ytes and fibrocytes, and a diminution of nutual coherence of the various tissue onents, as is clearly visible in Fig 2

art from these effects, no observable dif- e could be established between the iour of orthotopically transplanted anic membrane grafts preserved in vari- ways Neither the microscopic changes in raft nor the host reaction around the graft nfluenced by the type of preservation The ng process of the tympanic membrane was similar to that observed when fresh au- ous tissues are used (Reijnen & Kuijpers,) except for a very long persistence of the genous fibres of the tympanic membrane These appeared scarcely affected 1 1/2 years after transplantation (Fig 3) Grafts of fresh fascia or fat are usually absorbed or unrecognisable after the same period of time

These observations agree very well with the observations of MacKinnon (1972) and the case report of Marquet et al (1973) in which the persistence of the fibrous tissue of a cialit preserved tympanic membrane graft was demonstrated after 26 months

The tympanic membranes transplanted heterotopically into leg muscle also showed a marked resistance of the fibrous tissue against resorptive activity, irrespective of the method of preservation However, the persisting accumulation of mononuclear cells around the tympanic membranes preserved in formaldehyde is highly suggestive of a reaction to transplantation which can be explained by the fact that preservation in formaldehyde does not affect the transplantation antigens profoundly Similar observations were made previously around formaldehyde-preserved incus grafts transplanted in muscle A possible role of formaldehyde as a non specific stimulus causing this reaction was excluded (van den Broek & Kuijpers, 1974)

The behaviour of the malleus as part of the tympanic membrane/malleus graft does not dif-

fer from that of the incus grafts showing new bone formation in many cases after preservation in cialit and alcohol, but not after preservation in formaldehyde. No decalcification was observed in ossicles preserved in formaldehyde 4% (buffered at pH 7.0) as observed by Baron & Perkins (1971) using formaldehyde (buffered at pH 5.6). From the observations made after orthotopic transplantation of fresh heterologous ossicular grafts and of homologous ossicular grafts after previous sensitisation of the acceptor with tissue from the donor, the conclusion is allowed that graft rejection can occur in the middle ear. Similar observations have been made by Kastenbauer (1972). It therefore seems very likely that the absence of a transplantation reaction towards fresh homologous incudes in the middle ear is due to the small amount of tissue antigen transferred and the poor contact of the graft with the host tissue, as previously suggested (Kuijpers & van den Broek 1972). The same reasoning can be used to explain the absence of a reaction towards formaldehyde preserved grafts after orthotopic transplantation.

Although only a small number of human specimens could be studied, the observations done accord very well with the findings in animals. New bone formation was found in several ossicular implants which were preserved in cialit and alcohol and no signs suggestive of reactions to transplantation were found. Bone formation in the cialit and alcohol preserved grafts must be attributed to the preservation of the bone induction principle as defined by Urist et al. (1967). Comparable observations were made by Kerr & Smyth (1971) with alcohol preserved incudes. Yet apart from this partial remodelling which takes place in preserved ossicular grafts, limited areas of resorption and undeniable signs of bone replacement by fibrous tissue were registered in some of the human specimens (Figs. 11 and 12).

Although these data only represent selected cases, viz. specimens removed after failures but in the absence of inflammatory signs, the presence of large areas of [illegible] the replacement of bone by fibrous tissue [illegible] some question marks as to whether the [illegible] haviour of preserved ossicular [illegible] sorption of preserved bone [illegible] can occur to an even larger extent [illegible] with functional impairment. M[illegible] specimens will be necessary to ev[illegible] exact meaning of these findings.

ACKNOWLEDGEMENTS

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ZUSAMMENFASSUNG

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W Kuijpers M D
ENT Department
St Radboud Hospital
University of Nijmegen
Nijmegen
The Netherlands

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ERA-KENNLINIEN BEI NORMALHÖRENDEN PERSONEN

E Stürzebecher, K -D Wernecke und H Wagner

Aus der Hals Nasen Ohren Klinik des Bereichs Medizin (Charite) der Humboldt Universitat zu Berli dem Institut für medizinische Physik und Biophysik der Humboldt Universitat zu Berlin Berlin D

(Eingegangen am 25 November 1974)

Abstrakt Bei 28 normalhorenden Jugendlichen (20–25 Jahre) wurden die ERA Kennlinien und die entsprechenden Latenzverlaufe bei den Frequenzen 500 Hz 1000 Hz und 4000 Hz gemessen und in der Form A/μV uber HL/dB dargestellt Zur Untersuchung der Altersabhängigkeit der AEP wurde bei zwei weiteren Gruppen (30–50 Jahre und 55–70 Jahre) die 1000 Hz Kennlinie ermittelt Fur den Bereich 10–50 dB werden die Gleichungen der Regressionsgeraden angegeben Bei der Berechnung der Varianzen der Amplituden zeigte sich daß die intraindividuelle signifikant unter der interindividuellen Varianz liegt Prozentual als Variationskoeffizient ausgedruckt werden beide mit steigender Reizstarke geringer Fur die Latenzverlaufe werden die nach der Methode von Verhagen angepaßten Exponentialfunktionen angegeben Im Gegensatz zum Verhalten der Amplituden ergab sich bei den Latenzen keine signifikante Differenz der intra und interindividuellen Variationskoeffizienten Auch zwischen den Variationskoeffizienten der N1 und P2 Latenzen ist kein signifikanter Unterschied nachweisbar Bei mittleren Reizstärken weist die Variabilitat der Latenzen ein deutliches Minimum auf Eine Altersabhangigkeit der AEP im Bereich 20–70 Jahre ist nicht festzustellen

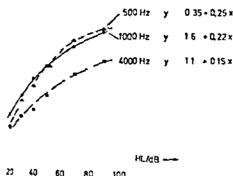
Fur die Einschatzung der Ergebnisse von Messungen mit der Evoked Response Audiometry (ERA) im uberschwelligen Bereich ist es notwendig, die Amplituden Kennlinien und Latenzverlaufe der evozierten Potentiale Normalhörender mit den entsprechenden Streubreiten vorliegen zu haben

Obwohl solche Kennlinien bereits von der Arbeitsgruppe um Stange (Muller & Stange, 1971, Benning et al , 1972 Stange, 1973) veroffentlicht wurden, haben wir diese Messungen nochmals durchgefuhrt da durch die von den genannten Autoren gewa stellung der Amplituden als „kortik gung" (die AEP Amplitude wird d aus einem reizunbeeinflußten Absc gemittelten EEG berechneten Be dividiert und nach Logarithmierung Maß als „kortikale Erregung" a (Spreng & Keidel, 1967)) die Üb dieser Normwerte sehr erschwert wi

METHODIK

Als Versuchspersonen (Vp) dien gesamt 28 normalhorende Jugendl Alter von 20–25 Jahren sowie zwei Altersgruppen (30–50 Jahre und 55–7 zu je 20 Vp Die Ermittlung der Kennl 500 Hz, 1000 Hz und 4000 Hz erfo einer Gruppe von 15 jugendlichen jeder Vp wurden pro Frequenz 4 Ke messungen an verschiedenen Tagen gefuhrt Zur Ermittlung der intraindiv Variabilitat wurde bei 13 jugendlichen Kennlinienverlauf bei 1000 Hz je 1(verschiedenen Tagen gemessen Fur stimmung der interindividuellen Va dienten die Messungen von allen 28 lichen Vp Bei den Altersgruppen 30– und 55–70 Jahre wurde zur Ermittl Altersabhängigkeit nur die 1000 Hz K gemessen

Die Dauer des Tonreizes war 500 10 ms Anstiegs und Abfallzeit (die A



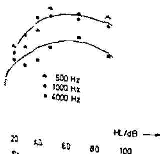
ERA Kennlinien für 500 Hz 1000 Hz und gemittelt über die individuellen Mittelwerte von 1 20–25jährigen Erwachsenen Die onsgleichungen gelten für den Bereich 10–50 dB

t in den 500 ms enthalten) Die Pausen- zwischen den Reizen war 2 s Das meter (PAR 140) ist nach ISO Norm ert Mit diesem Audiometer wurde vor Messungen auch die subjektive Hor- le der Vp ermittelt Bei allen Vp war eichung der individuellen Hor le bei den 3 Frequenzen von der ISO le kleiner als 5 dB Die Mitteilung mit einem Averager (DL 102 A, ALAB) durchgeführt, der eine auto- Artefakt Unterdruckung ermog Jeweils 64 Einzelantworten wurden elt Der EEG Verstarker (PAR 113) nen Eingangswiderstand von 100 MΩ, Grenzfrequenz war 30 Hz, die untere 0,3 Hz Die durch diese Bandbegrenzung bewirkte Verzogerung des Signals liegt nach unseren Messungen bei 2 ms Da die meisten Autoren mit ahnlichen Bandbegrenzungen arbeiten und die Verzögerung gering ist, haben wir die gemessenen Latenzen nicht mit diesem Wert korrigiert Eine Beeinflussung der AEP-Amplitude ist bei den gewahlten Grenzfrequenzen noch nicht nachweisbar Die Ableitung erfolgte mit der standardisierten Elektrodenlage Vertex gegen Mastoid des unbeschallten Ohres und Erdung der Stirn Die Vp lasen wahrend der Ableitung Texte nach eigener Wahl Als Amplitude des AEP wird die N1–P2 Differenz bezeichnet Alle statistischen Berechnungen wurden auf einem LINC-8 durchgefuhrt

ERGEBNISSE

Abb 1 zeigt die ermittelten Kennlinien bei den Frequenzen 500 Hz, 1000 Hz und 4000 Hz Die Kennlinien bei 500 Hz und 1000 Hz verlaufen praktisch gleich, bei 4000 Hz sind die Amplituden des AEP jedoch wesentlich geringer Die in Abb 1 angegebenen Regres sionsgleichungen wurden fur den Bereich 10 dB bis 50 dB berechnet

Zur besseren Ubersichtlichkeit wurden die Standardabweichungen der Amplituden in Abhangigkeit von der Reizstarke gesondert in Abb 2 dargestellt



Standardabweichungen der Amplituden aus Abb 1 von der Reizstarke

3

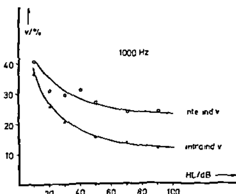


Abb 3 Intra und interindividuelle Variationskoeffizienten *v* der AEP Amplituden in Abhängigkeit von der Reizstarke ($v = (s/M \cdot 100\%)$)

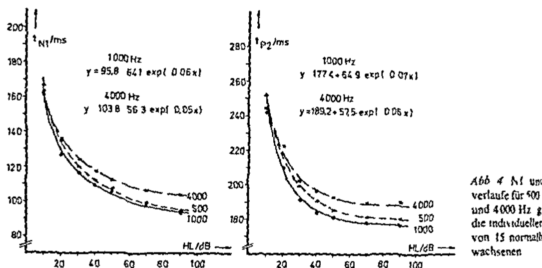


Abb 4 N1 un
verlaufe für 500
und 4000 Hz g
die individueller
von 15 normalh
wachsenen

Fur 1000 Hz wurden die intraindividuelle und die interindividuelle Standardabweichung getrennt ermittelt und in Abb 3 als Variationskoeffizient $v = (s/M) \cdot 100\%$ (s=Standardabweichung M=Mittelwert) in Abhängigkeit von der Reizintensitat dargestellt Der intraindividuelle Variationskoeffizient ist der Mittelwert der bei 13 Vp aus jeweils 10 Potentialen pro Reizintensitat berechneten individuellen Variationskoeffizienten Die interindividuellen Variationskoeffizienten wurden aus den Abweichungen der individuellen Mittelwerte vom Gesamtmittelwert bestimmt [1] Im gesamten Intensitatsbereich liegt die interindividuelle Variabilitat der Amplitu[...] fikant über der intraindividuellen

Die zu den AEP Amplituden d[...] gehorenden Latenzen zeigt Abb 4 [...] Hz wurden die kurzesten Latenzen [...] Sowohl fur N1 als auch P2 liegt die [...] Latenzkurve im Bereich 20–90 dB [...] signifikant uber der 1000 Hz Kurve [...] 1% Niveau darunter 5% Niveau) [...] ferenzen zwischen 500 Hz und 100[...] nicht signifikant (Uberprufung mit [...] sischen t Test) Fur die 1000 Hz und [...] Kurven sind in der Abb 4 die [...] Methode von Verhagen (Rasch & [...] berger, 1967) angepaßten Regre[...] chungen angegeben Die Stand[...] chungen der Latenzen sind ebenfall[...] seren Übersichtlichkeit gesondert [...] dargestellt

Wie fur die Amplituden so wurde[...] die Latenzen bei 1000 Hz die intra [...] individuellen Variationskoeffiziente[...] hangigkeit von der Reizstarke [...] Abb 6 zeigt diese Variationskoeffiz[...] die Latenzen von N1 und P2 Im [...] zu den Amplituden sind jedoch be[...] tenzen keine Differenzen zwischen [...] interindividueller Variabilitat nach[...] Abb 7 enthalt die 1000 Hz Kenn[...] 3 Altersgruppen 20–25 Jahre 30–50 [...] 55–70 Jahre Die Differenzen der K[...] sind nicht signifikant

[1] Damit berechnet sich der intraindividuelle Variationskoeffizient zu

$$v_{intra} = \frac{1}{k} \sum_{i=1}^{k} v_i$$

$$\text{mit } v_i = \frac{s_i}{\bar{x}_i} \cdot 100\%, \quad \bar{x}_i = \frac{1}{n} \sum_{j=1}^{n} x_{ij}$$

$$s_i^2 = \frac{1}{n-1} \sum_{j=1}^{n} (x_{ij} - \bar{x}_i)^2$$

Den interindividuellen Variationskoeffizienten erhalt man aus der Formel

$$v_{inter} = \frac{s}{\bar{x}} \cdot 100\%$$

$$\text{mit } \bar{x} = \frac{1}{n \cdot k} \sum_{i=1}^{k} \sum_{j=1}^{n} x_{ij} \quad \text{und} \quad s^2 = \frac{1}{k-1} \sum_{i=1}^{k} (\bar{x}_i - \bar{x})^2$$

In unserem Fall gilt stets $k = 13$, $n = 10$

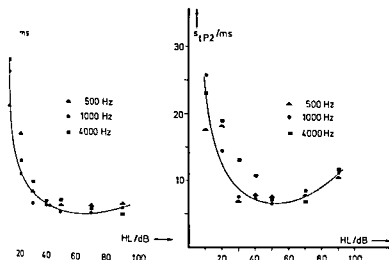


Abb 5 Standardabweichung *s* der Latenzen von Abb 4 in Abhängigkeit von der Reizstärke

DISKUSSION

nlinien

die Kennlinien wird im Gegensatz zu eren Autoren (Spreng & Keidel, 1967, ller & Stange, 1971, Benning et al , 1972) direkte Darstellung der Amplituden in μV r der Reizintensität in dB bevorzugt Die rstellung der AEP Amplitude als kortikale regung wie sie von Spreng & Keidel (1967) geschlagen wurde, hat neben Vorteilen rminderung der Streuung) auch Nachteile Bezugsgröße für die Berechnung der korti en Erregung die aus einem reizfreien schnitt ermittelt wird, ist abhängig vom quenzgang des Verstärkers, wodurch die rgleichbarkeit der Ergebnisse erschwert rd Ihre Berechnung von Hand ist zeit bend und fehleranfällig Die maschinelle rechnung erfordert einen freiprogram erbaren Rechner Da die Reizstärke hängigkeit der AEP Amplituden offen ich nicht einer Potenzfunktion gehorcht, rbt der Verlauf der kortikalen Erregung ch keine Gerade über den interessierenden tensitätsbereich 0–90 dB Andererseits ist folge der komplexen zentralen Verarbeitung ch kein linearer Zusammenhang zwischen r Reizstärke in dB und der AEP Amplitude μV zu erwarten Da jedoch für den praktischen Gebrauch ein lineares Modell Vorteile besitzt, wurde mit Hilfe eines statistischen Linearitätstestes nach Draper & Smith (1967) getestet ob zumindest in dem Bereich 10 dB bis 50 dB eine lineare Abhängigkeit der Amplituden von der Reizstärke in dB anzunehmen oder abzulehnen ist Voraussetzung für die Anwendung dieses Testes ist das Vorliegen mehrerer Meßwerte pro Abszissenwert

Die Anwendung des Testes sowohl auf die Mittelwertskurven bei den 3 Frequenzen als auch auf die individuellen 1 000 Hz Kennlinien der 15 Vp ergab nur für 1 Vp Ablehnung

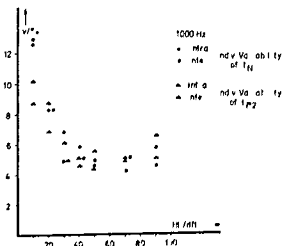


Abb 6 Intra und interindividuelle Variationskoeffizienten der N1 und P2 Latenzen in Abhängigkeit von der Reizstärke

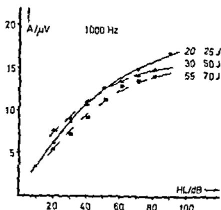


Abb 7 1000 Hz Kennlinien der Altersgruppen 20–25 Jahre 30–50 Jahre und 55–70 Jahre

des linearen Modells, für die anderen 14 individuellen Kennlinien sowie für die Mittelwertskurven der 3 Frequenzen wird im Bereich 10–50 dB ein linearer Verlauf nicht abgelehnt

Um die Darstellung als kortikale Erregung zu simulieren, wurden alle Amplitudenwerte, die zur Berechnung der Mittelwertskurven dienten, logarithmiert und wieder getestet Bei allen 3 Frequenzen ergab sich daß ein linearer Zusammenhang zwischen Reizintensitat in dB und der kortikalen Erregung in dB auch in dem engen Bereich 10–50 dB abgelehnt werden muß Das ist als weiterer Nachteil der Darstellung der AEP Amplituden als kortikale Erregung zu werten

Frequenzabhangigkeit der Amplituden

Der Anstieg der Regressionsgeraden fur 4000 Hz ist erwartungsgemaß deutlich geringer als bei den tieferen Frequenzen Auf Grund ihrer Messungen stellten Davis et al (1968) die Hypothese auf, daß bei gleicher Lautstärke auch bei unterschiedlichen Reizfrequenzen annahernd gleiche Antwortamplituden resultieren Die geringeren Amplituden bei hoheren Frequenzen, verglichen mit 1000 Hz, waren dann mit einer geringeren Lautstarke zu erklaren Da die Kurven gleicher Lautstarke jedoch zwischen 1000 Hz und 6000 Hz annahernd parallel verlaufen (ISO/R 131–1959), konnen die kleineren Amplituden bei hohen Frequenzen bei gleichen dB W[…] uber der Hörschwelle in diesem Bereic[…] unserer Ansicht aber nicht mit ein[…] ringeren Lautstarke in Zusammenha[…] bracht werden

Als Ursache dieser Frequenzabhän[…] der evozierten Potentiale ist die unters[…] liche Breite der Reizverteilung auf der B[…] membran (Kohlloffel, 1972) und dam[…] unterschiedliche Anzahl der aktiv[…] neuronalen Einheiten bei hohen und […] Frequenzen wahrscheinlicher (Antino[…] Skinner, 1968)

Variabilitat der Amplituden

In den Variationskoeffizienten der Abb […] sowohl der Versuchsfehler als auc[…] tatsächliche Variabilitat enthalten De[…] stieg der Kurven bei kleinen dB Wert[…] sultiert daher zu einem Teil sicher aus […] großeren biologischen Streuung der A[…] tuden bei geringeren Reizstarken […] anderen Teil aber beeinflussen Mittel[…] fehler die kleinen Amplituden bei ger[…] Reizstarken relativ starker Die Ausme[…] wird schwieriger und ist mit einem gro[…] relativen Fehler behaftet

Die von Karnahl & Stange (1972[…] gegebene Abhangigkeit des Variationsk[…] zienten von der Reizintensitat (ohne […] spaltung in intra und interindividuelle V[…] bilitat) weist prinzipiell denselben Verlau[…] jedoch liegen die Werte dieser Autoren […] gesamt niedriger Die Ursache dieser […] ferenz ist eventuell die von Spreng & K[…] (1967) angegebene Verminderung […] Streuung durch Umrechnung der A[…] Amplitude in kortikale Erregung D[…] mußte dann vor allem die interindivid[…] Streuung vermindert werden deren Ante[…] der Gesamtstreuung wesentlich großer ist[…]

Um eine statistisch gesicherte Aus[…] darüber machen zu konnen ob die Poten[…] verschiedener Personen einer Grundgesa[…] heit mit entsprechend großer Varianz z[…] ordnet werden konnen oder ob sie u[…]

chen Populationen zuzuordnen sind, eine Varianzanalyse mit den 130 werten der 13 Vp bei 50 dB durchgeführt Varianzanalyse ergab mit einer Irrtumsichkeit von 1% Signifikanz, d h entiale der einzelnen Personen unterden sich wesentlich

dieser Stelle muß betont werden, daß alle gemachten Aussagen nur auf die ituden beziehen, nicht auf den i Potential Zeitverlauf Eine solche lyse wird zur Zeit durchgeführt

Vergleich der Ergebnisse in Abb 4 mit von Stange (1973) veröffentlichten Hz Latenzverläufen zeigt gute Über der N1 Latenzen Das gilt für P2 Latenzen nicht Während Stange im 20–80 dB eine Verkürzung der Latenz etwa 65 ms feststellte, konnten wir nur mehr als 30 ms messen Im Bereich dB ändern sich die P2-Latenzen nach 4 nur noch geringfügig, bei Stange i noch um mehr als 20 ms

as von Benning et al (1972) gefundene eau der 1000 Hz N1 Latenz zwischen 40 60 dB bestätigen unsere Messungen nicht Verfasser fanden auch eine andere ängigkeit der Latenzen

ährend nach Abb 4 die 500 Hz Latenzen mit den 1000 Hz Latenzen überein und sich signifikant von den 4000 Werten unterscheiden, fanden Benning (1972) keine Unterschiede zwischen den i bei 500 Hz und 4000 Hz

bei beiden zitierten Arbeiten anzufallsverteilte Reizung im Gegen zur regelmäßigen Reizung bei den vorden Meßwerten kann die erwähnten nicht erklären, da nach unseren ngen (Sturzebecher et al 1974) bei hem mittleren Reizintervall keine wesent Latenzdifferenzen bei regelmäßiger zufallsverteilter Reizung auftreten

Altersabhängigkeit

Aus Abb 7 ist ersichtlich, daß die Amplituden des AEP im untersuchten Altersbereich praktisch keine Altersabhängigkeit aufweisen Dasselbe gilt auch für die hier nicht abgebildeten Latenzen

SUMMARY

The ERA characteristics (amplitudes and latencies) were measured in normal hearing adults (20 to 25 years of age) at frequencies of 500 Hz 1000 Hz and 4000 Hz Two other age groups (30 to 50 and 55 to 70 years of age) were measured only at 1000 Hz Contrary to other authors the linear scale was chosen to represent the



significant differences of amplitudes and latencies between the age groups could be found

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Dipl -Ing E Sturzebecher
HNO Klinik der Humboldt-Universität
Schumannstr 21
104 Berlin, DDR

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MELANOCYTEN, LANGERHANSSCHE UND MERKELSCHE ZELLEN IN ORALEN EPITHELIEN

P Schenk

Aus der Hals Nasen Ohren Klinik des Karolinska Sjukhuset Stockholm und dem Konig Gustav V Forschungsinstitut Stockholm Schweden

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lr Die feinstrukturelle Morphologie von Melano Langerhansschen und Merkelschen Zellen im nor Epithel der dorsalen Zungenschleimhaut des hen wird elektronenmikroskopisch dargestellt ocyten werden im normalen Papillenepithel regel vorgefunden wenn klinisch auch keine Zeichen Pigmentierung vorliegen Der basale epitheliale ocyt enthalt individuelle Pigmentorganellen ver en der Melaninbiosynthese und ent t rudimentare Halbdesmosomen gegen die Basal ran. Die morphologischen Besonderheiten der durch spezifische Organellen sowie das von Tonofilamenten und Desmosomen gekenn en Langerhansschen Zellen deuten auf funk e Beziehungen zu Zellstrukturen der Keratinocyten epithelialen Keratinisationsprozeß Die Mer Zellen sind durch ihre horizontale Basallage te zu Keratinocyten Fehlen von typische Merkelsche Granula und an Nervenendigung charakterisiert. Die beobach usion von Merkelschen Granula mit dem peripheren der Merkel Zellen und die morphologische aft dieser Granula mit den Monoamin la bestimmter chromaffiner Zellsysteme Hinweise auf eine symbiotische Einheit von rter Merkelscher Zelle und assoziiertem Neu mit neuroepithelialer Mechanorezeptorfunktion kelschen Zellen erscheinen aufgrund ihrer spezi Ultrastruktur als selbstandige von Keratinocyten dendrit schen Zellen unabhangige Zellpopulation Epithelien

mehrschichtige Plattenepithel keratini- r Mundhohlenregionen stellt ahnlich der s eine symbiotische Einheit dar die er 90 % aus Keratinocyten aufgebaut ist

Zu den intraepithelialen Nicht-Keratinocyten zahlen Melanocyten, Langerhanssche und Merkelsche Zellen Letztere Zellpopulation, von Merkel als „Tastzellen" beschrieben (Merkel, 1875), ist erst in jüngster Zeit in das Blickfeld elektronenmikroskopischer Betrachtungen geruckt Aufgrund ihrer Goldaffinitat wurden die Langerhansschen Zellen (Langerhans, 1868) eine lange Zeit fur neurogene Elemente gehalten Nach Abtrennung von den Zellen des pigmentbildenden Systems, die erst in den letzten Jahren erfolgte, wird nunmehr eine mesenchymale Herkunft dieser Zellpopulation diskutiert Funktion und biologische Dignitat der Langerhansschen Zellen sind jedoch bis heute unbekannt

Die Ultrastruktur der Keratinocyten des Papillenepithels der menschlichen Zunge ist an anderer Stelle (Schenk & Wersall, 1975) dargestellt worden In der vorliegenden Arbeit werden elektronenmikroskopische Beobachtungen uber das Vorkommen und die feinstrukturelle Morphologie von Melanocyten, Langerhansschen und Merkelschen Zellen im normalen Epithel der dorsalen Zungenschleimhaut des Menschen mitgeteilt

MATERIAL UND METHODEN

Biopsien normaler Schleimhaut der dorsalen Zungenoberflache wurden von zehn Patienten, ohne Vorgeschichte oder klinische

Unterstutzung durch Fonds des Karolinska Institutet kholm

Zeichen einer Erkrankung der Mundhohlenschleimhaut, in Lokalanasthesie (Xylocain 0 5 %, Astra) entnommen

Die Gewebeproben zur lichtmikroskopischen Untersuchung wurden in neutraler, gepufferte 7 % iger Formaldehyd Lösung fixiert, in Paraffin eingebettet und mit Hama toxylin Eosin gefarbt

Die Praparate zur elektronenmikroskopischen Untersuchung wurden in kalter 1 % iger Osmiumtetroxyd Lösung, gepuffert mit Vero nalacetat auf pH 7 4, fixiert, dehydriert und in Epon eingebettet 1–3 μm dicke Schnitte wurden mit Toluidinblau gefärbt und mit dem Lichtmikroskop orientiert Ultradunnschnitte (300–500 Å) wurden mit einem LKB Ultramicrotom angefertigt und auf Kupferobjekttrager montiert *Die Nachkontrastierung erfolgte* mit Uranylacetat (Watson, 1958) und mit Bleicitrat (Reynolds, 1963) Die elektronen mikroskopische Untersuchung der Praparate wurde mit einem Siemens Elmiskop I bei einer *Strahlspannung von 80 kV durchgeführt*

Melanocyten

Melanocyten kommen als regelmaßiger Bestandteil des Zungenepithels zur Beobachtung, wenn klinisch auch keine Anzeichen einer Pigmentierung gegeben sind (Abb 1*a*) Der Kern dieser dendritischen Zellen ist meist weniger tief eingebuchtet, als dies bei den Langerhansschen Zellen der Fall ist, und kann bisweilen eine mehr abgerundete Form an nehmen Die charakteristische Organelle dieser Zellpopulation und zugleich Endpro dukt der Melaninbiosynthese ist das ca 0 3 μm×0 1 μm große, je nach Schnittebene runde, ovale oder langliche Melanosom mit *homogenem elektronendichten Inhalt und* außerer, nicht immer erkennbarer Mem branbegrenzung Die Pramelanosomen die als Vorstadien der Melanosomentwicklung gelten, besitzen eine granulare, spiralige oder striäre Innenstruktur mit einer 90–100 Å Periodizitat und einer dem Reifegrad der Melanogenese entsprechenden Elektronendichte (Abb 1*b*) Wahrend der A[illegible] zum Melanosom kommt es durch zu[illegible] Pigmentablagerung zu einer Maskie[illegible] periodischen Innenstruktur der Gra[illegible] melanosomen werden von dem C[illegible] der Melanocyten bzw ihrer za[illegible] Dendriten in das Cytoplasma der [illegible] barten Keratinocyten transferiert [illegible] als individuelle Pigmentorganellen [illegible] Form von mindestens 2–3 Melanoso[illegible] haltenden, membranbegrenzten [illegible] somenkomplexen vorgefunden

Das kontrastarme Cytoplasma der [illegible] cyten enthalt neben zahlreichen R[illegible] und Mitochondrien spärlich individu[illegible] plasmatische Filamente geringer El[illegible] dichte und ohne Neigung zur [illegible] *bundelung Golgi Apparat und* [illegible] matisches Reticulum sind gut aus[illegible] Der in den basalen und suprabasale[illegible] vorkommende Melanocyt ist gegen d[illegible] membran häufig durch cytoplasmatis[illegible] *laufer der angrenzenden Keratinoc*[illegible] gedeckt Desmosomale Verbindun[illegible] Keratinocyten oder anderen dend[illegible] Zellen kommen nicht vor, gegen d[illegible] membran konnen jedoch modifizier[illegible] desmosomartige Strukturen an der pr[illegible] Zellgrenze entwickelt werden

Langerhanssche Zellen

Langerhanssche Zellen kommen im [illegible] epithel der menschlichen Zunge re[illegible] im Stratum spinosum vor und besitze[illegible] wie die Melanocyten die ultrastru[illegible] Merkmale der entsprechenden dend[illegible] Zellen der Epidermis (Abb 2*a*) Das [illegible] mentfreie Cytoplasma dieser dend[illegible] Zellen ist meist kontrastarmer als das [illegible] grenzenden Keratinocyten und enth[illegible] viduelle cytoplasmatische Filamente [illegible] lich denen des Melanocyten keine [illegible] zur Aggregation aufweisen Neben [illegible] chondrien, Ribosomen, einem gut a[illegible] deten Golgi Apparat und endoplasma[illegible] Reticulum finden sich Lysosomen [illegible] und Vakuolen, Lipideinschlusse und [illegible]



1a Melanocyt mit zahlreichen Pigmentorganellen der Basalzellreihe des dorsalen Zungenepithels. *N* Zell-, *S* Sphaeridion, *G* Golgi-Zonen, *ICR* Interzellular-, *BM* geschichtete Basalmembran, *KC* Keratinocyten mit cytoplasmatischen Ausläufern

Abb. 1b Prämelanosomen in verschiedenen Schnittebenen mit granulärer, spiraliger oder striärer Innenstruktur und deutlich erkennbarer dreischichtiger membran. Die Elektronendichte der Pigme variiert entsprechend dem Reifestadiu

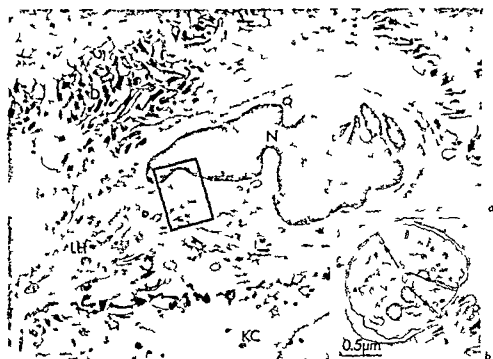
Zeichen von mitotischer Aktivitat Die fur diese Zellenart typischen Organellen sind die bis zu 1 μm langen, stabförmigen Langerhansschen Granula, die von parallelen Membranen begrenzt werden und eine zentrale doppelreihige Lamelle sowie je eine randstandige einreihige Lamelle elektronendichter 50 Å-Partikel mit einer 90 Å-Periodizität enthalten (Abb 2*c*) Bei dem Tennisschlagertyp der Langerhans-Granula besitzt das Endstück einer Seite eine vesikulare Erweiterung Bei flachem Anschnitt der in der dritten Dimension schalen- oder scheibenförmigen Gebilde wird eine parakristalline Innenstruktur sichtbar (Abb 2*c*) Langerhanssche Granula wurden im Papillenepithel vereinzelt in Kontakt und auch in Kontinuitat mit der Plasmamembran gefunden (Abb 2*b*) Der langliche, eher grazil gebaute Zellkern besitzt die für diese Zellenart charakteristischen tiefen Einbuchtungen seiner Kernmembran

Desmosomale Zwischenzellkontakte zu Keratinocyten oder anderen intraepithelialen Zellen werden nicht entwickelt Cytoplasmatische Vorstülpungen der Langerhansschen Zellen in entsprechende Invaginationen der angrenzenden Keratinocytenmembran sind jedoch haufig nachweisbar und konnten fur einen intensiven funktionellen Kontakt zwischen den beiden Zellpopulationen sprechen Langerhanssche Zellen fanden sich haufig in unmittelbarer Nahe von interzellularen Ansammlungen mikrovilloser Cytoplasmafortsatze, die mit zahlreichen Desmosomen behaftet waren (Abb 2*a*) Bei Kontaktaufnahme mit Langerhansschen Zellen kam es in den peripheren Cytoplasmaabschnitten benachbarter Keratinocyten haufig zu einer Retraktion und Invagination von Desmosomen sowie zu einer massiven Konzentration und raumlichen Ausrichtung von cytoplasmatischen Tonofilamenten An anderen Stellen wurden hingegen umschriebene tonofilamentfreie, organellen und kontrast arme, periphere Cytoplasmazonen in an grenzenden Keratinocyten beobachtet Die Anzahl der Langerhans-Zellen war in starker keratinisierten Regionen deutlich groß dem an das Papillenepithel grenzende gangsepithel mit geringer Verh tendenz

Merkelsche Zellen

Die Merkelsche Zelle liegt mit ihrer achse meist parallel zur elektron skopischen Basalmembran und ist ge häufig durch Cytoplasmafortsätze barter Keratinocyten abgegrenzt (A Den Merkel-Zellen ist intraepithelia eine diskusartige myelinfreie Nerven mit zahlreichen Mitochondrien und angelagert Regelmaßiger findet man Nervenendigungen im subepithelialen gewebe in unmittelbarer Nahe d kelschen Zelle Der langliche Zellken oft tiefe Invaginationen der Kernmem Cytoplasma der Zellen, das in sein trastarmut den beschriebenen dend Zellen vergleichbar ist, verlaufen fe plasmatische Filamente, die keine zur Aggregation aufweisen Das ul turelle Erkennungszeichen sind d kelschen Granula, 600–1500 Å groß branbegrenzte, rundliche bis ovale Das elektronendichte Innere dieser ist von der Außenmembran durch e förmige kontrastarme Zone getrenn 3*b*) Größe, Zahl und Elektronendi Granula variieren vermutlich entsp einem unterschiedlichen Funktion Reifezustand Granulaformen mit Durchmesser an der unteren Gre genannten Größenordnung besitzen eine kontrastarmere granular fila Innenstruktur und/oder einen exzen inneren Kernanteil Gelegentlich sind einer Kontaktaufnahme oder Fusi Granula mit dem peripheren Plasmal beobachten (Abb 4*a* *b*) Bei polar richtung der Merkel-Granula in basal lateralen Zellabschnitten ist der Golgi an der diesen Regionen abgewendete des Zellkernes gelegen

Die voluminosen Mitochondrien,





• Lange hanssche Zelle (*LH*) m t t ef e nge Zellkern (*N*) und dendn schem Cytoplasma- (*DT*) m S r sp no um des Pap llenep hels zte mikro ose Ep thelzellfortsa ze In erzel ularraum angrenzende Kera nocyten

2b Dendnt e ner I angerhans Zelle m Q schn tt Langerhanssche Granula (Pfe le) n Kontakt m t der Plasmamembran

Abb 2c Ve großerte Ausschnı t aus Abb *2a* Stab förm ge Langerhan sche Granula (*LG*) m t penod nnerer Lamellen n ktur Parakn all ne (Pfe le) be flachem Anschn t der Zellkern

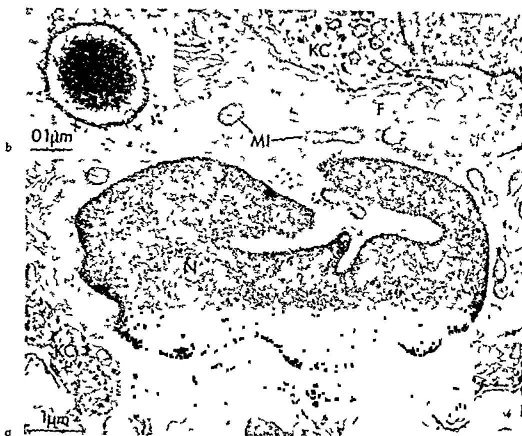


Abb 3a Merkelsche Zelle in typischer horizontaler Basallage Eingebuchteter Zellkern (*N*) voluminose Mitochondrien (*MI*) Merkelsche Granula (Pfeile) und cytoplasmatische Filamente (*F*) Keratinocyten (*KC*) mit Cytoplasmafortsätzen Basalmembran (*BM*) an der Bindegewebsgrenze

Abb 3b Merkelsches Granulum bei ... größerung Der elektronendichte innere ... von granular filamentoser Beschaffenheit ... Elemente erstrecken sich in d e kontrast ... und erreichen an manchen Stellen d e ... ferenz der dreischichtigen Außenmembran

maßig geringer als in Keratinocyten, zahl reiche Ribosomen Partikel und granulares endoplasmatisches Reticulum sind im Cyto plasma verteilt Daneben findet man oft zahl reiche cytoplasmatische Vesikel multivesi kulare Korper Lysosomen und vereinzelt Centriolen In der Cytoplasmaperipherie sind stellenweise Zeichen einer hohen pino cytotischen Aktivitat vorhanden An der la teralen und distalen Plasmamembran sind vereinzelt schwach ausgebildete desmosom artige Kontakte zu Keratinocyten nachweis bar Die mit der Haftplatte der desmosomalen Strukturen assoziierten Filamente sind aus serst sparlich ausgebildet oder fehlen zur Ganze Zellverbindungen zu dendritischen Zellen oder anderen Zellen als Keratinocyten sowie zur Basalmembran werde... wickelt

Übrige intraepitheliale Zellen

Neben den erwahnten Zellpopula... den im Papillenepithel der Zunge ... dendritischen Zellen ahnliche k... Zellen in basaler und suprabasal... getroffen die in ihrem Cytoplasm... beschriebenen spezifischen Orga... halten Es könnte sich dabei un... geschilderten Zelltypen handeln den ... raktenstische Organellen außerhalb der ... trachteten Schnittebene oder in dendriti... Zellfortsatzen lokalisiert sind wo sie ... durch Serienschnitte nicht immer aufzude... sind Andererseits konnte sich die betreff...



Fusion eines Merkelschen Granulums mit dem ...m der Merkel Zelle Vergroßerter Ausschnitt 4b

... Merkelsche Zelle mit Merkel-Granula (*MG*) ... Abbildung obere Granulum in Fusion mit der Plasmamembran Eingebuchteter Zellkern (*N*) voluminose Mitochondrien (*MI*) *ICR* Interzellularraum Durch Serienschnitte konnten in dieser Zelle weitere Merkel-Granula und Desmosomen aufgedeckt werden

...sche Zelle in einem Entwicklungs-...unktionszustand befinden, bei dem ...ifischen Organellen tatsachlich nicht ...ken oder mit den verwendeten praparat...ethoden nicht nachweisbar sind Falls ...ber nicht beschriebene dritte Art von ...schen Nicht Keratinocyten existieren ...so liegen bislang keine ausreichenden ...ukturellen Kriterien vor, die eine ...zierung einer solchen Zellpopulation ...n wurden

Vorkommen von intraepithelialen Ner...igungen wurde bereits oben erwahnt ...halb des Niveaus der Merkelschen Zel...den sich freie Nervenendigungen im ...ellularraum oder in Plasmamembranin...tionen von Keratinocyten Das hoch...isierte Epithel der Geschmacks...ragenden Zungenpapillen kann hier ...wahnt werden Die Einwanderung von verschiedenen Zellen wie Lymphocyten, Granulocyten, Makrophagen usw aus dem subepithelialen Bindegewebe ist auf Einzel beobachtungen beschrankt und kann eher als die Ausnahme zur Regel gelten Das intraepitheliale Auftreten von Mastzellen, das unter pathologischen Bedingungen vorkommen kann, ist ebenfalls kein regelmäßig zu beobachtendes Charakteristikum des normalen Papillenepithels der menschlichen Zunge

DISKUSSION

Uber Melanocyten, Langerhanssche Zellen und Merkel Zellen des Papillenepithels der menschlichen Zunge liegen bisher keine elektronenmikroskopischen Untersuchungen vor Hutchens et al (1971) untersuchten die Verteilung der dendritischen Zellen in oralen

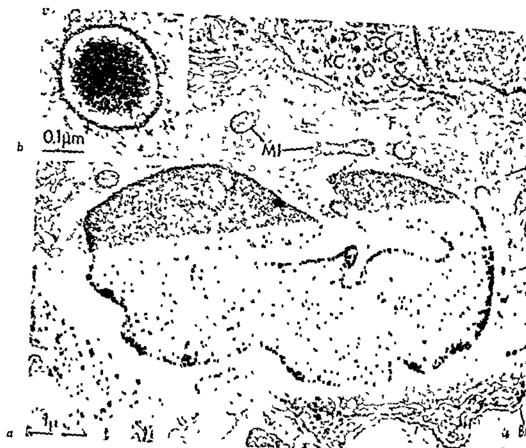


Abb 3a Merkelsche Zelle in typischer horizontaler Basallage Eingebuchteter Zellkern (*N*), voluminöse Mitochondrien (*MI*), Merkelsche Granula (Pfeile) und cytoplasmatische Filamente (*F*), Keratinocyten (*KC*) mit Cytoplasmafortsatzen, Basalmembran (*BM*) an der Bindegewebsgrenze

Abb 3b Merkelsches Granulum bei s*
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Ubrige intraepitheliale Zellen

Neben den erwahnten Zellpopulatio den im Papillenepithel der Zunge hä dendritischen Zellen ähnliche, kor Zellen in basaler und suprabasaler L getroffen, die in ihrem Cytoplasma k beschriebenen spezifischen Organell halten Es könnte sich dabei um e geschilderten Zelltypen handeln, der rakteristische Organellen außerhalb trachteten Schnittebene oder in dendr Zellfortsatzen lokalisiert sind, wo s durch Serienschnitte nicht immer aufzu sind. Andererseits konnte sich die betre

. und bei Vitiligo (Ebner & Niebauer, ınd von uns in Plattenepithelcarcinomen öffentlichte Befunde) regelmäßig ge- i Ein dreidimensionales Modell der rhans-Granula wurde von Wolff (1967*b*) fen Kontakt oder Kontinuität der la mit der Plasmamembran wurde in fällen in unserem Material gesehen 2*b*), viel häufiger war jedoch eine Kon t mit Golgi Elementen gegeben, was auf ntstehung aus dem Golgi Apparat hin- i könnte (Zelickson, 1965, Wolff, 1967*b*, & Schreiner, 1970) Von anderen en wurde, zum Teil ebenfalls auf Tracer- nzmarkierungen gestutzt, eine ent- gesetzte, i e zentripetale Wanderungs- ng und ein endocytotischer Entste- mechanismus der Langerhans Granula Abschnurung eingestulpter Plasma- ananteile postuliert (Tarnowski & moto, 1967, Hashimoto & Tarnowski, Hashimoto, 1970)

r spezifische Ultrastruktur der Langer- chen Zelle deutet auf eine aktive, zur e fähige und mit Spezialfunktionen stattete Zelle Ein funktioneller Zusam ang mit dem Keratinisationsprozeß wird serem Material durch Vermehrung der erhans Zellen in Epithelien mit höherem rnungsgrad deutlich In unverhornten helien der Ratte wurden Langerhans- n nach Vitamin A Entzug gleichzeitig dem Auftreten von keratinproduzieren Metaplasien gefunden (Wong & Buck, Beim Rhesus-Affen war in den kera- rten Epithelien der Mundhöhle die An- der Langerhans Zellen höher als in nicht- rnten Epithelien und am größten in dem ornten Epithel des Zungenrückens chens et al , 1971) Eine Phagocytose- tion scheint nicht die Haupteigenschaft Langerhansschen Zellen zu sein Wir en am pathologischen Material (unver- ntlichte Befunde) phagocytierte Zellbe steile von Langerhans Zellen im Cyto ma benachbarter Keratinocyten Eine entliche Funktion dieser Zellen erscheint uns über eine Wirkung auf Desmosomen, Tonofilamente und Zellmembranen von Keratinocyten eine Beeinflussung des Differenzierungs- und Keratinisationsprozesses verhornender Epithelien zu sein

Die Merkelschen „Tastzellen", denen mechanorezeptorische Funktionen zugeschrieben werden, kommen in der Basalschicht des Papillenepithels vereinzelt oder in Gruppen von mehreren Zellen vor Kontrastarmes Cytoplasma, Merkel-Granula, Desmosomenkontake und angelagerte Nervenendigung sind charakteristische Merkmale dieser Zellen Kurze, zapfenartige Cytoplasmaprozesse erstrecken sich zwar gegen angrenzende Keratinocyten und breitbasige Cytoplasmavorstülpungen gegen andere, benachbarte Merkel Zellen, die Ausbildung von langen, dendritischen Zellfortsätzen ist jedoch nicht zu beobachten, weshalb die Merkelschen Zellen nicht als dendritische Zellen bezeichnet werden können

Über Merkel-Zellen im Epithel der Gingiva wurde von Hashimoto (1972) berichtet, „gut entwickelte Bündel von Tonofibrillen" konnten wir in den Merkel-Zellen unseres Materials nicht beobachten Von Fortman & Winkelmann (1973) dargestellte filamentose Kerneinschlüsse sind nach unseren Befunden nicht kennzeichnend für diese Zellenart, sie konnten jedoch Hinweise auf eine neurogene Herkunft der Merkelschen Zellen geben Ähnliche Gebilde wurden in Zellen des Cerebralganglions von Enchytraeus nach Puromycingabe gesehen (Gersch & Ude, 1971) Das Vorkommen einer angelagerten Nervenendigung im Niveau der Merkelschen Zelle wurde bei verschiedenen Säugetieren und beim Menschen beschrieben (Munger, 1965, Mustakallio & Kiistala, 1967, Smith, 1970, Suzuki & Kurosumi, 1972), ist aber in der oralen Mucosa (Fortman & Winkelmann, 1973) zumindest kein regelmäßig zu beobachtendes Charakteristikum dieser Zellpopulation

Eine Fusion der Merkelschen Granula mit dem Plasmalemm (Abb 4*a*, *b*) konnte als morphologisches Anzeichen einer Entleeru

und Abgabe des Granulainhaltes in den extrazellulären Raum gedeutet werden Die einzigen Hinweise auf eine Fusion der Merkel-Granula mit der Plasmamembran ergaben sich bisher in den dermalen Grandryschen Korperchen von Embryonen der Pekingente (Saxod, 1970) und in der Buccalmucosa der Ratte (Chen et al , 1973) Den Merkelschen Granula ahnliche „dense-cored vesicles" konnten kurzlich in neuroepithelialen Körperchen der Bronchial- und Bronchiolarmucosa von Kaninchen gefunden und ihr Gehalt an Serotonin nachgewiesen werden (Lauweryns et al , 1973)

Die morphologische Verwandtschaft der Merkelschen Granula mit den Catecholamingranula der Zellen des Nebennierenmarkes, sympathischer Ganglien und anderer chromaffiner Gewebe, sowie der Nachweis von synaptischen Strukturen in Merkelschen Endigungen verschiedener Mammalia (Andres, 1966, Halata, 1973, Chen et al , 1973) deuten auf eine funktionelle Einheit von spezialisierter Merkelzelle und assoziierter Nervenendigung Mechanische Impulse, die auf das Epithel einwirken, würden dabei durch Cytoplasmafortsatze und Desmosomen von der Merkelschen Zelle aufgenommen, transformiert und durch extrazellulare Abgabe einer Transmittorsubstanz aus den Merkel-Granula auf die sensorische Nervenendigung ubertragen

Die Bestatigung dieser Hypothese sowie die Aufklarung der chemischen Beschaffenheit der Merkelschen Granula muß physiologischen, pharmakologischen, mikrospektrographischen und elektronenmikroskopisch-cytochemischen Untersuchungen vorbehalten bleiben Die spezifische Ultrastruktur dieser Zellenart veranlaßt uns zu der Auffassung, daß die Merkelschen Zellen eine selbstandige, von Keratinocyten und dendritischen Zellen unabhangige Zellpopulation oraler Epithelien darstellen

Für ihre wertvolle technische Assistenz sei Frl Ann Marie Lundberg und Herrn Bengt Hedberg herzlich gedankt

SUMMARY

The fine structural morphology of melanoc[...] hans cells and Merkel cells in the normal dorsa[...] of the human tongue has been studied by ele[...] scopy Melanocytes were regularly found in [...]

[...]

turation and rudimentary half-desmosomes [...] between the melanocyte and the basal mem[...] suprabasal Langerhans cells can be identifi[...] specific organelles and by the lack of tonofila[...] desmosomes The morphological features of [...] suggest functional relations to cell structures [...] tinocytes and to the epithelial keratinization p[...] Merkel cell is characterized by a horizontal [...] in the basal epithelial layer the development [...] somal attachments to keratinocytes the lack [...] ments the typical Merkel cell granules and an[...]

[...]

epithelial tactile receptor function Because of [...] fic ultrastructure, the Merkel cells are thou[...] stitute a self maintaining cell population inde[...] keratinocytes and dendritic cells in the oral [...]

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Dr Peter Schenk
Konig Gustav V Forschungsinstitut
S 104 01 Stockholm 60
Schweden

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SURVIVAL AFTER HAEMORRHAGE FROM THE BRACHIOCEPHALIC TRUNCUS FOLLOWING TRACHEOSTOMY

R Adolfsson, B Winblad and Y Ostberg

From the Department of Pathology and Otolaryngology University of Umeå Umeå Sweden

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Abstract A patient with two massive haemorrhages from the brachiocephalic truncus after tracheostomy is reported An analysis of 6 previously reported long term survivors following this complication shows that the initial care in controlling the haemorrhage is of major importance The operation method of choice is permanent division of the brachiocephalic truncus Transitory or no neurological postoperative complications were found

The most dangerous and dramatic complication after tracheostomy is the delayed, massive haemorrhage from the brachiocephalic truncus Of all the complications following tracheostomy, this unforeseen catastrophe has the highest mortality rate (Mathog et al , 1971) Considering the increased number of tracheostomies performed, resulting from the widening indications for tracheostomy, the frequency of this complication is on the increase (Bihler & Hutschenreuter, 1966, Comer et al , 1974) Not until 1968, was the first long-term survivor reported by Reich & Rosenkrantz (1968) To the best of our knowledge, only 6 patients have previously been recorded as surviving after a haemorrhage from the brachiocephalic truncus and after leaving hospital

We thought it worthwhile to report on a patient who survived two such haemorrhages but later expired In order to contribute to a better prognosis for patients suffering from this kind of haemorrhage an analysis was made both of the case and the , ported long term survivors C stances, which may have contr survival, are discussed

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Case report

The patient was a 60-year-old wo pled by advanced rheumatoid ar with a pronounced kyphoscolios admitted to the hospital suffering spiratory stridor and increasing resp sufficiency due to complete paraly left vocal cord and partial paralysis c vocal cord Tracheostomy was p She received a Portex tracheost which was exchanged for a silver ca one week The silver cannula had placed by a Rüsch tube, because of a of the trachea, which caused a part termittent obstruction of the silver Several times the tube was dislodged tracheostoma due to violent coughi

Nine months after the tracheos performed, a slight haemorrhage w from the tracheostoma However, spontaneously A sudden, unexpec sive haemorrhage occurred 4 days l the tracheostomy tube was routinely The tube was immediately reintrod cuff was inflated and the haemorrh be controlled An erosion of the cephalic truncus was now suspe the patient was taken to the operat



Fig 1 Frontal view of the superior thoracic outlet The aortic arch (*A*) is displaced to the left The brachiocephalic truncus (*T*) leaves the aortic arch dislodged to the left of the midline and transverses abnormally high in the thoracic cavity The close relationship between the inferior concave side of the tracheostomy tube and the brachiocephalic truncus is seen

tracheostoma was carefully inspected ...nly a new massive haemorrhage ap... This could temporarily be controlled ...ital compression through the lower part ... tracheostoma against the sternum An ...racheal intubation was performed during ...al anaesthesia and a median sternotomy ...arried out This created a good access to ...spected area of haemorrhage A defect ...granulations was found in the wall of the ...iocephalic truncus, near the lower bor...f the tracheostoma, where the truncus ...he trachea were in close spatial relation to each other The defect in the vessel was sutured The tracheostoma was ...ned upwards and the cricoid cartilage was ...ed to create a better lodge for the tracheo...ny tube A Portex tracheostomy tube inserted The postoperative course was ...mplicated

...rteen days later another sudden massive ...norrhage from the tracheostoma occurred ...h could be temporarily controlled by immediate inflation of the cuff At operation a new erosion of the vessel in the formerly sutured area was found After resection of a small necrotic part of the vessel wall, the defect was closed by sutures No division of the artery was done and no graft was inserted Postoperatively the patient was treated by artificial respiration through an endotracheal tube She died 10 days later due to respiratory and circulatory insufficiency

Autopsy

Macroscopical findings A pronounced kyphoscoliosis in the thoracocervical region with several collapsed vertebral bodies were found Moderate to severe arteriosclerosis was noticed in the coronary vessels The myocardium showed slight fibrosis In the pericardial sac, fibrin adherences were found The lungs liver, spleen and kidneys showed a massive blood congestion

The lower border of the tracheostoma was in close relationship to the brachiocephal



Fig 2 Low power photomicrograph showing a portion of the mucosa (*M*) and cartilage (*C*) of the trachea and the elastic membranes (*E*) in the wall of the brachiocephalic truncus The elastic fibres are split up and the arterial wall is replaced by granulation tissue (*G*) Thrombotic material is adhering to the luminal surface of the granulation tissue van Gieson-elastin × 10

truncus which left the aortic arch to the left of the midline and crossed the trachea at an abnormally cranial level (Fig 1) At the cranial surface of the vessel approximately 3 cm from the aortic arch disintegrating blood clots and granulations covered the sutured area No communication was found to the lumen of the artery On the corresponding intimal surface of the vessel wall thrombotic material was found

Microscopical findings Granulation tissue and loose connective tissue with scattered inflammatory cells were found between the brachiocephalic truncu and the tracheal wall in the sutured area In the vessel wall degeneration of the elastic fibr vas seen In some parts the vessel wall was otally necrotic and replaced by granulation tissue At the luminal surface of the granulation issue a thrombus was present (Fig 2)

DISCUSSION

Almost every case of massi haemorrhage as a complication I tracheostomy presents as a complet to the clinician A fulminating ha may occur at any time but seldom one month after the tracheostomy Hutschenreuter 1966 Brantigan 1 case has been reported occuring postoperatively (Weissman 1974) Th cephalic truncus is the most com volved vessel (Bihler & Hutschenre Biller & Ebert 1970) No patient gr said to be predisposed to this serio cation It is of greatest importanc how to handle this type of haemorr 50% of the patients exsanguinate airways in seconds if an immediat the haemorrhage is not obtained Ebert 1970)

In our case the haemorrhage a late as 9 months after the tracheost ously the thoracic deformity caus placement of the aortic arch res abnormal cranial position of th cephalic truncus Anomalous p major vessels has previously bee as a cause in the pathogenesis of th tion (Scheldrup 1957 Wel n 1964 1973) Jarvis (1964) reported a pati origin of the brachiocephalic trunc of the midline similar to that rec patient Occasionally the truncus the level of the second and third (Willerson 1965) and the close the inferior concave surface of ostomy tube thus predisposes to caused by constant pounding o against the tracheostomy tube (B 1970 Utley et 197?) A simila has obviou reflects th lower t (Silen

A nec

[Table] I Review of 6 previously reported long-term survivors after massive erosion haemorrhage [from th]e brachiocephalic truncus following tracheostomy

[Indir]ect erosion means a mechanism of vascular erosion caused by the tip of the tracheostomy tube with necrotizing [ulcer]ation and secondary penetration through the tracheal wall Direct erosion means a mechanism of vascular [erosion] caused by a constant pounding of the vessel wall against the inferior concave surface of the tracheostomy tube

..	Patient	Symptoms prior to haemorrhage	Post operative interval (days)	Pathogenesis	Initial care	Operative method	Comment
	14 year old girl	None	225	Indirect erosion	Cuff inflation	Sternotomy division	No neurological complications
	70-year old woman	Hemoptysis	16	Direct erosion	Tube reinsertion digital compression endotracheal intubation	Sternotomy division	Haemorrhage at decannulation no neurological complications
	21 year old girl	None	9	Direct erosion	Tube reinsertion	Sternotomy division	Haemorrhage at decannulation slight weakness of the arm
	11 year old girl	None	7	Direct erosion	Endotracheal intubation digital compression	Sternotomy division	No neurological complications
	11 year old girl	None	6	Direct erosion	Digital compression tube reinsertion	Suprasternal approach division	No neurological complications
an	27 year old man	None	540	Direct erosion	Tube reinsertion digital compression	Sternotomy division	Haemorrhage at decannulation no neurological complications

[d]ue to an iterating trauma by the tip of the This type of vascular erosion is regarded [be]ing the most important mechanism in [formin]g a tracheo-arterial fistula (Lunding, Reich & Rosenkrantz, 1968, Mathog et [al 19]71) Only one patient has been reported [sur]viving this type of arterial erosion (Table [Rei]ch & Rosenkrantz, 1968)

[Th]e classical warning symptoms prior to [a m]assive haemorrhage are of importance A [...]self limiting haemorrhage a few days [befor]e the major haemorrhage has been re[porte]d (Rogers, 1969, Biller & Ebert, 1970, [...]er 1973) This was also noticed in the [...] reported here A pulsating tube (Wil[lerson] 1965), and a feeling of suprasternal dis[comf]ort together with an irritating cough can [als]o be present before a massive haemorrhage [(N]ordmollin, 1962)

[Th]e frequency of serious, delayed [haem]orrhages induced by erosion of the [brach]iocephalic tr[uncus] (Rogers, 1969, Kantawala et al, 1972) At the University Hospital of Umeå, Sweden, 1000 tracheostomies have been performed during the last 10 years and 2 cases of tracheostomy tube erosion haemorrhage have been registered However, this figure probably is too small since a fistula between the trachea and the artery can be rather difficult to find, not only at operation, but also at autopsy (Willerson 1965, Weissman, 1974)

The total incidence of all types of haemorrhage after tracheostomy is about 1–3% (Rogers, 1969, Mathog et al, 1972, Kantawala et al, 1972) The early postoperative slight oozing of blood from the tracheostoma is due to inadequate hemostasis (Willerson, 1965, Rogers, 1969) Only a few hours after a tracheostomy, a developing dry haemorrhagic tracheobronchitis can be observed (Pfretzschner et al, 1973) Concerning the sudden massive haemorrhage from a

[...]ning different

diagnostical considerations should be avoided All postoperative haemorrhages should be looked upon as if they were prodromal signs of early erosion of a major neck vessel

A review of the 6 cases of long-term survival after haemorrhages from the brachiocephalic truncus (Table I) shows that the haemorrhages started at the time of decannulation in 3 cases By analysing the initial care of these 6 survivors, it can be recommended to recannulate the patient immediately, if the haemorrhage occurs by the time of decannulation Inflation of the cuff should also be done According to Utley et al (1972), the initial inflation of the cuff is unsuccessful in controlling the haemorrhage in most patients, but likewise the initial procedure should be a cuff inflation in order to prevent aspiration If it is not possible to cuff the tube or control the haemorrhage by inflation of the cuff, insertion of the index finger through the tracheostoma is recommended, in order to compress the brachiocephalic truncus against the sternum After this an endotracheal intubation can be performed (Table I) Restoration of blood volume prior to operation is of great importance to reduce postoperative neurological complications, which are expected to appear in 20–30%, when using the recommended operation method with division of the brachiocephalic truncus (Biller & Ebert 1970) However, a minor postoperative neurological complication was reported in only one of the long term survivors Thus the apparent operative method of choice after a median sternotomy is permanent division of the truncus (Table I) This method was used in all long term survivors

ZUSAMMENFASSUNG

Ein Patient mit zwei massiven Blutungen aus dem Truncus brachiocephalicus nach einer Tracheostomie ist beschrieben worden Eine eingehende Analyse von sechs früher beobachteten Überlebenden zeigt dass eine frühzeitige und adäquate Kontrolle der Blutung von grosser Bedeutung ist Die gewählte Operationsmethode ist eine endgültige Teilung des Truncus brachiocephalicus Vorübergehende oder neurologisch postoperative Komplikationen waren nicht vorhanden

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Y Ostberg M D
Dept of Otolaryngology
University of Umeå
S 901 87 Umeå
Sweden

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HE PROBLEM OF UNILATERAL AND BILATERAL NECK DISSECTION

Z Krajina, S Simović and S Večerina

From the Department of Otolaryngology University of Zagreb Medical Faculty Zagreb Yugoslavia

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270 unilateral and bilateral neck resections have rformed in different primary localisations of in the head and the neck at the Ear Nose and Department of the Medical Faculty in Zagreb in od 1960–70 Of 209 patients with unilateral resec ly 78 survived (37%) while only 3 patients (4 9%) 1 of a total of 61 patients where bilateral resection formed An analysis is given separately of radical n in carcinoma of the larynx the hypopharynx and t. From this analysis and from immunologic con ons the following conclusions are derived (i) The are not in favour of prophylactic block resection would be contrary to our knowledge of the role by the immunobiological factors in the struggle the malignant lesion (ii) Evacuation of the neck performed in carcinoma of the larynx T_3 and in aryngeal carcinoma as histologically positive nodes are usually found in this site in large s

roblem of radical neck dissection be a focus of attention in 1906 when Crile shed his work Excision of the Head and ' in America A large number of cases reviewed in this work, i e 4500 patients ring from carcinoma of the head and neck had died because of an inadequately ed primary tumour or metastases in the while only 1% of deaths were due to nt metastases The hope aroused by otherapy slightly checked the enthusiasm surgery but it very soon became apparent metastases in the neck were resistant to liation and in a great number of cases the entage of recoveries was accordingly low refore the advocates of surgical therapy prevailed once more, whereby the American authors MacComb (1969), Martin & Morfit (1944), and Conley (1967) contributed most and radical neck resection became the subject of interest not only for otorhinolaryngologists but for a large group of head and neck surgeons too

Moreover it became not only the method of choice but a sort of prophylaxis against the spreading of carcinoma, because some authors as, e g Willis (1952) maintained that the lymph nodes not only are no obstacle to but even represent fertile soil for the development of carcinoma Thus in the course of time an ever increasing number of authors in the ear, nose and throat branch of medicine began to favour the idea that, e g , in laryngeal carcinoma as the most frequent malignant localization it is necessary to carry out parallel block resection of the neck in all cases except in localizations on the vocal cords

Twenty two out of a number of 38 otorhinolaryngologists from different countries were in favour of this solution in an enquiry conducted by the Milan School of Medicine prior to the International Congress of Paris in 1961 The first report on radical neck resection in Yugoslavia was given by our Department in 1956 at the Congress for Maxillofacial and Plastic surgery, when it was pointed out that we could not possibly favour prophylactic block resection since by removing the l

tissue of the neck it was not possible to change the relationship between the malignant lesion and the immunologic forces of the organism Subsequently our standpoint was strengthened as, by studying the relationship between the immunoglobulins and the malignant tumour, we discovered that each malignant lesion inevitably engaged the entire defensive system and that on this relation alone the problem of prophylactic block resection should be viewed

Over the last few years immunology rapidly became involved with the problems of cancer and we have now valuable data at our disposal concerning this sphere of activity

CLINICAL OBSERVATIONS AND IMMUNOBIOLOGICAL ASPECTS

On the basis of the present-day investigations in anatomy and physiology it may be concluded that the lymphatic system of the neck is part of the general lymphatic defence system of the organism which, due to its localization in the anatomical sense, holds a specific position for the organs of the head and the neck

While the entire lymphatic system of the other parts of the body is directed towards the thoracic duct and the right lymphatic duct, the lymph from the region of the head and the neck flows towards the supraclavicular fossae and it is therefore clear why, in contrast to the other localizations, metastases in the region of the neck remain in this site for a longer period before passing into the region of the mediastinum or into other organs as distant metastases

The spreading of the tumour from the primary focus into the lymphatic nodes is embolic in character, thus between the tumour and the metastases there is no continuity of malignantly altered lymphatic ducts It was also realized that the tumour does not spread from one lymph node to the other but unifocal or multifocal spreading from the primary lesion is present from the very beginning Multifocal spreading marks a greater discord between the tumour and the organism clinical experiences have taught us forms are highly malignant It is necessary to undertake a histo of all removed lymph nodes in every radical resection

Siegel & Fisch (1965) showed that ical resection of the neck the lymp via the subcutaneous and the nodes on the same and the contrala Welsh & Welsh (1963) and Pressm (1960) showed that following block r the neck, contralateral lymph usually appears In 1947 Furuta regeneration of the lymph nodes whereby an important role was at the youth of the experimental an histological investigations (Krajina in cases of radical neck resection in the fatty and fluffy tissue of stretching from the subcutaneous ti deep musculature of the neck, small tions of lymphoreticular elements ent, which can subsequently d smaller or larger lymph nodes The which develop after radical resectio frequently located subcutaneously deep structures of the neck, leadin complications

The following experiments also role of the lymphatic apparatus in against malignant tumours

Crile (1969) studied the relation b tumour and the regional lymph no planted S-180 into the heel of a observed the course of the disea moval or irradiation of the lymph n popliteal region If these nodes tirpated within a period of 4 to 10 implantation of the tumour, not e duced resistance against the tumou a higher incidence of pulmonary developed too Extirpation of the ly 10 days after implantation did not f firm this dependence Besides he a ter results with ordinary mastecto patients in the first stage of a tum

-A than by radical resection of the ** and the regional lymph nodes
clinical experiences might serve to cor- e these results of Crile's investigations -/ the development of regional ses after two or more years and with- al recurrence would indicate the defen y-le of the regional lymph nodes The -e of enlarged lymph nodes, histologi- negative, on the side of the primary r moreover indicates the participation of -paratus in the defence against the ant process A more marked lympho- eaction in the environs of the tumour is -ive of a better outcome of the malignant

-ay it is established without any doubt e lymph nodes take part in the forming -oral immunoglobulin elements via their , itic elements and by means of the -ocytes in the creation of sessile and -ting cellular antibodies which play an tant role in the development of the late le reaction While in malignant diseases moral antibodies maintain their high re for a long period, with the develop- of the disease the lymphocytic reaction ses rapidly Thus we could establish that % of our patients a decrease in the er of lymphocytes in the blood below was a sign of the fatal outcome of the e

ne 15 years ago we established that in ts suffering from malignant tumours in RL region the quantity of immunoglobu howed an increase, though this higher uld not indicate a less malignant lesion, on the contrary indicated the increased er, to the organism and that only in the l stage the immunoglobulin quotient d to decrease somewhat
ver the last few years it was moreover d that malignant tumours develop more uently after transplantation of organs be e of the application of immunodepressant s and since then immunol gy has begun to etrate more and more into the problems of malignant disease Eilber & Morton (1970) showed that in 93% of all their patients with a late negative reaction to DCNB (dinitrochlorbenzene) the prognosis was poor Negative tuberculin reaction also appears in the majority of late cases The organism reacts to foreign substances through the lymphocytes, from which such notions are then transmitted to the next few generations The antigen capacity of tumours has so far been noted especially in malignant melanoma, osteogenic sarcoma and Burkitt's lymphoma Testing at our clinic the capacity of the organism to react to its own tumour in the same way as to a foreign antigen, we were unable to observe any legality between this reaction and the prognosis of the disease On the contrary we were able to establish a very small number of positive reactions The observation, however, that in 90% of all patients with very malignant tumours the number of lymphocytes in the blood falls below 10% is no doubt a very significant symptom in the prognosis, and at the same time an indicator of the general immunobiological state in combating this malignant disease

All these examples and clinical observations confirm that the lymphopoietic apparatus is not a passive track alone over which the malignant cells slide, but a defensive apparatus by means of which the general defensive power of the organism against the malignant process is expressed

CLINICAL MATERIAL

For the study of the problem of unilateral and bilateral resection of the neck we selected the clinical material covering the period 1960 to 1970 but only of those patients in whom classic complete unilateral or bilateral neck resection had been performed We considered that for this problem chosen for the subject of our study it might be sufficient to take into consideration carcinoma of the larynx, hypopharynx and the skin While in cases of laryngeal

Table I *Radical neck dissection performed in 108 cases of laryngeal cancer (1960–70)*
RP=recurrence of the primary tumour
RM=recurrence of the metastases
Distant metastases lung 3 cases

	Living	Dead	Unknown	Total
Unilateral neck dissection				
T_1N_1	2	1 RM 1	2	5
T_2N_1	13 RP 1 RM 1	5 RP 1 RM 4	3	21
T_3N_1	20 RM 2	28 RP 10 RM 24	7	55
T_4N_1	–	3 RP 1 RM 2	1	4
	35	37	13	85
Bilateral neck dissection				
T_1N_2	–	–	1	1
T_2N_2	–	–	–	–
T_3N_2	3 RM 1	10 RP, 3 RM 8	2	15
T_3N_3		1 RP, 1 RM 1	–	1
T_4N_2		5 RP 2 RM 5	–	5
T_4N_3	–	1 RM 1	–	1
	3	17	3	23

carcinoma we kept in that period to the principle of carrying out therapeutical block resection only in cases of carcinoma of the hypopharynx we have been performing preventive block resection of the neck and extirpation of the primary tumour in the same act The lymph nodes of the neck extirpated because of carcinoma of the larynx were histologically positive in all our patients and in carcinoma of the hypopharynx negative in one case only

I In laryngeal carcinoma (Table I) we achieved recovery in 35 of 85 patients (41%) by means of unilateral neck resection while in cases where bilateral resection had to be applied this ratio was 3 23 thus amounting to a mere 13% A separate analysis of t
tumours in group T_1 and T_2 show
percentage of recoveries in case
primary tumours was higher (15
57% The analysis of the surviva
tients with a primary tumour n
showed a recurrence of the prima
only one of our 38 cases while
developed in 4 cases out of these 3
was significantly changed in th
deaths where the primary tumour
18 of 54 cases while regional
showed recurrence in 46 out of

II In carcinoma of the larynx
pharynx (Table II) or in carcinor
the hypopharynx (Table III) the pe
recoveries was considerably low
patients with unilateral metasta
(24%) survived while of the 16 p
bilateral metastases none survived
tients with this localization of t
tumour who survived, there was
case of recurrence of the primary t
was there recurrence of the
metastases These results are in n

Table II *Radical neck dissection pe 23 cases of laryngeal and hypop cancer*
Distant metastases lung 1 case

	Living	Dead	Unk
Unilateral neck dissection			
T_4N_0		1	–
T_4N_1	3	6 RP 3 RM 5	1
T_4N_3	1	–	–
	4	7	1
Bilateral neck dissection			
T_3N_2		8 RP 3 RM 7	
T_4N_2		3 RP 1 RM 3	–
		11	–

III *Radical neck dissection performed*
cases of hypopharyngeal cancer

…t metastases lung 5 cases vertebral column 1

	Living	Dead	Unknown	Total
…ral neck dissection				
	–	–	1	1
	2	–	2	4
	1	2 RP, 1 RM 2	1	4
	–	3 RP 1 RM, 3	–	3
	–	5 RP, 3 RM 5	–	5
	3	10	4	17
…al neck dissection				
	–	1 RM 1	–	1
	–	4 RP 2 RM 4	–	4
	–	5	–	5

… to the results in the group of deaths in … recurrence of the primary tumour was in 14 of 33 patients and metastases in 30 patients Distant metastases developed in …geal carcinoma in 3 of 108 patients (3%) …n 7 of 45 patients (16%) in carcinoma of …ypopharynx

Our analysis included carcinomas of the … of the head and the neck which were …arily treated by irradiation and usually … a somewhat better prognosis than the …r localizations of carcinoma of the head …the neck Of the 12 patients however, in …m regional metastases had developed, 11 … Excluding from this group two …anomas which *a priori* are highly malignant …gional metastases develop, this high lethal …rate in carcinoma of the skin of the head …the neck with regional metastases clearly …ws that the appearance of metastases is a …n of weakening of the immunological forces …the organism

DISCUSSION

We believe that on the basis of our clinical studies which particularly deal with carcinoma of the larynx, hypopharynx and the skin of the head and the neck, we may draw some conclusions about the value of resection in general, about the relationship between bilateral and unilateral resection of the neck and of the value of prophylactic resection

Although with regard to block resection our attitude in carcinoma of the larynx is generally therapeutic and carcinoma of the hypopharynx prophylactic, the final results clearly indicate that the malignancy of the tumour and the relationship of the lymphatic system towards the degree of malignancy are of decisive importance for the outcome of the disease Thus, for instance, laryngeal carcinoma even subsequent block resection of a smaller tumour gives good results, while in carcinoma of the hypopharynx in spite of the prophylactic resection of the neck the results are markedly poor Naturally it is wrong to speak of prophylactic block resection in carcinoma of the hypopharynx if, in spite of the negative palpatory finding, the presence of metastases is proved histologically

Invasiveness of carcinoma of the hypopharynx vis-à-vis carcinoma localized in the hypopharynx and the larynx, leads much more rapidly to destruction of the defensive powers of the organism, just the same as the deeper position of the metastases in such a primary localization leads to infiltration of the vagus nerve and the carotid artery Not a single one of our patients with infiltration of the vagus nerve or the carotid survived Carcinoma of the skin was taken as an example of a tumour which metastasizes However, the appearance of metastases in carcinoma of the skin indicates marked invasiveness of the tumour and a weakening of the immunobiological forces of the organism in the struggle against the malignant lesion The following conclu are derived from these considerations

We are not in favour of prophy

resection, since this would be contrary to our knowledge of the role played by the immunobiological factors in the struggle against the malignant lesion

Evacuation of the neck must be performed in carcinoma of the larynx T_3 and in hypopharyngeal carcinoma because histologically positive lymph nodes are usually found in this site in large numbers

The great hope expressed by Crile in 1906 that surgical therapy would solve the problem of carcinoma of the head and the neck has so far not come true The high percentage of recurrences after unilateral and especially after bilateral resection of the neck indicates that other therapeutic means must also be applied in the struggle against malignant growths in the head and the neck

Radical neck resection has been performed in 270 patients—unilateral in 209 and bilateral in 61 of our patients Of the 209 patients in whom unilateral resection was performed 78 (37%) survived, while of the 61 patients in whom bilateral resection had to be performed only 3 (4 9%) survived

The organism engages in the struggle against the malignant lesion with its immunobiological apparatus The first defeats of the organism in this struggle are reflected by the appearance of unilateral metastases, are continued by the development of bilateral metastases, and finally the organism and its defence mechanism break down completely with the appearance of distant metastases Compensation and recovery may occur in unilateral metastases, much less frequently in bilateral and only exceptionally in cases where distant metastases have developed

ZUSAMMENFASSUNG

An der Hals Nasen Ohren Klinik der Medizinischen Fakultät in Zagreb wurden von 1960 bis 1970 270 unilaterale und bilaterale Halsresektionen bei verschiedenen primären Lokalisationen [illegible]

wird eine besonderte Analyse der radikalen Res[illegible] Larynx Hypopharynx und Hautkarzinome[illegible] Auf Grund dieser Analyse und der [illegible] Betrachtungen folgen die Autoren. Eine [illegible] Blockresektion wird nicht befürwortet, weil [illegible] Erkenntnissen von der Rolle der [illegible] Faktoren im Kampf mit der malignen Läsion [illegible] Bei Larynxkarzinom T_3 wie auch bei [illegible] karzinom soll die Halsevakuation doch [illegible] werden weil hier bei einem grossen Prozents[illegible] die Lympfknoten histologisch positiv sind

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Z Krajina M D
Dept of Otolaryngology
University of Zagreb Medical Faculty
Šalata 4
Zagreb
Yugoslavia

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INHIBITORY PROCESSES IN THE MEDIAL GENICULATE BODY

B Etholm

rom the Institute of Neurophysiology University of Oslo and the Department of Oto-rhino-laryngology Ulleval Hospital Oslo Norway

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t Inhibition in the medial geniculate body was with a double click technique A conditioning click a marked inhibition of field potentials and unit ges to a subsequent test click Cyclic inhibition period of about 150 ms was seen in the medial ite body and the auditory cortex but not in the r colliculus The postsynaptic excitability was by recording from the killed ends of the thalamo- fibres A preceding click reduced the direct e to a test stimulus delivered to the medial ate body indicating the presence of postsynaptic n The excitability of the fibres from the inferior us terminating in the medial geniculate body was with a modified Wall (1958) method The results the presence of a presynaptic inhibitory mech m the medial geniculate body The techniques ed do not allow an estimation of the relative ation of the two inhibitory mechanisms to the on at the medial geniculate level

auditory nerve fibres respond to loud ls over a large frequency range With just above threshold, however, they to a distinct frequency Plotting of the d threshold versus frequency will, there give a wide angled tuning curve The ted peak of the tuning curve represents haracteristic frequency (CF) of the fibre aki 1954 Kiang et al 1965) At higher ls of the auditory nervous system the ng curves are progressively narrower, and reported to be most contracted at the medial geniculate (MG) level (Katsuki et al, 1959*a*) The explanation for this phenomenon may be sought in part by the prominent inhibitory processes found at various levels in the auditory pathways Thus, Whitfield (1955), recording from units in the trapezoid body, found a distinct reduction of the response to the second of two tones compared with the response to a single tone of the same frequency and loudness Similarly, Katsuki et al (1959*b*) found that spontaneous as well as tone evoked discharges of single units in the higher levels of the auditory pathway were suppressed when a background tone was delivered simultaneously with the test signal The degree of the suppression was dependent upon the level of the background sound or noise Refining these observations Nomoto et al (1964) found that the most effective reduction was produced by a conditioning tone having a frequency either just above or just below that of the test tone

Aitkin et al (1966) have shown that spontaneously active units in the medial geniculate body are suppressed for 40 to 200 ms after a click In their work the possibility of both pre- and postsynaptic inhibition in the MG, modulating the responses to sound stimuli was discussed, but not tested However, postsynaptic potentials (IPSPs) have been recorded from MG neurones (Nelson &

investigation has been supported by the Norwegian arch Council for Science and the Humanities and the ersity of Oslo

Erulkar, 1963) Excitability studies following stimulation of the brachium of the inferior colliculus have demonstrated long lasting inhibition, however, without distinguishing between pre- and postsynaptic inhibitory mechanisms (Aitkin & Dunlop, 1969) In view of the presence of both pre- and postsynaptic inhibition in other parts of thalamus (Andersen et al , 1964, Burke & Sefton, 1966), a similar arrangement in MG seems possible

The aim of the present investigation has been to study the time course and degree of the click-induced inhibition found in MG and to study the mechanisms underlying the inhibition In particular, the possibility that presynaptic inhibitory mechanisms could be present in addition to the earlier known postsynaptic inhibition, was tested It will be shown that presynaptic depolarization, which is associated with inhibitory processes in other parts of the central nervous system (Eccles, 1969) operates in MG

METHODS

Adult cats were anaesthetized with 30 mg sodium pentobarbital per kg, given intraperitoneally Maintenance doses (10 mg/kg) were given intravenously to maintain a moderate light barbiturate anaesthesia, characterized by a withdrawal of the foreleg on pinching of the paw The head was fixed in a head holder with ear bars The bar to the test ear was hollow In some animals with wide bony auditory canals the bars had to be pushed so far in to fix the head that the ear drums were damaged In such animals the sound was increased by 15–20 dB but the shape of the input/output curve recorded from the medial geniculate was similar to that normally observed

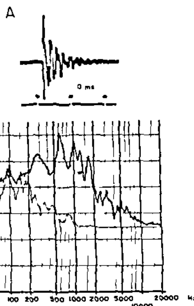
The skull overlying the dorsal part of the brain was removed and the skin flaps were sewn to a ring to prepare a pool which was filled with warm liquid paraffin The dorsal part of the thalamus was exposed by removing the overlying neocortical tissue of the lateral and suprasylvian gyri and the hippocampus

The MG body is situated only 6-9 m[m] the dorsal surface of the thalamus B[y] approach the surface of the inferior co[lliculus] could be seen directly The exposure [facili]tated accurate placement of the re[cording] electrodes By comparing the results w[ith and] without cortical removal no sig[nificant] change of the responses could be note[d in] the tests applied

Extracellular recordings of unit a[ction] potentials were made by glass capillari[es filled] with solutions of 4 M NaCl or 3 M K[Cl. The] micro electrode signals were fed to a c[athode] follower and displayed on an oscilloscop[e after] a c amplification To counteract vari[ability of] the responses, particularly with regard [to the] tendency of rhythmic activity in the th[alamus,] a computer of average transients was [used in] part of the study

Surface records from the auditory [cortex] were made with small platinum ball ele[ctrodes] (0 5 mm diameter) Recordings fr[om the] "killed ends" of the thalamo-cortical [fibres] were made from the white matter un[der] the primary auditory cortex by using a [...] thick platinum wire insulated with po[lythe]lene except at the tip and cut off sq[uare.] After the cortex had been sucked aw[ay the] electrode was moved over the surface [of the] white matter in order to find the [...] recording point Larger signals were o[btained] by pushing the electrode approximately [...] into the white matter When properly [...] the "killed ends" recording appears as [a posi]tive monophasic deflection Care was t[aken to] remove all surrounding auditory cor[tex to] avoid possible contamination with c[ortical] field potentials The signals from the [white] matter or the auditory cortex were a[mplified] and displayed on an oscilloscope

Clicks were elicited by feeding [square] pulses of 50 μs duration to an ampl[ifier that] drove a loudspeaker Clicks were pref[erred to] tones because excitability studies requ[ire de]livery of the sound stimulus with abrupt [onset.] The click had a sharp initial transie[nt and] evoked a recognizable response at all le[vels]



(A) Recording of a click of 108 dB (B) Frequency t of the same click (heavy line) analysed in 3 dB ncy bands Thin line gives the background noise of perimental room The amplitude is sound pressure e 0 0002 dyn/cm²

auditory nervous system (Rosenzweig & nblith 1953) It was delivered by the peaker to a funnel feeding a tube The h of the tube from the loudspeaker to the w bar inserted into the external auditory was 56 cm and had a bore of 7 mm The was connected to a Y shaped air pipe a hollow bar attached to each end The were 10 cm long with a bore of 3 mm One was inserted into the external auditory l whereas the other was connected to an icial ear consisting of a rubber tube oppos linear condensor microphone The cavity lined with soft material to imitate the soft s of the external and middle ear and its ume measured 1 3 cm³

Measured in the artificial ear the shape of a k with an amplitude of 108 dB is shown in 1 A The oscillations are heavily damped h an interpeak period of approximately 1 7 corresponding to a frequency around 600 the amplitude of the second wave usually measured less than 60% of the first The frequency content of the click is given in Fig 1 B (heavy line) showing a large peak at 620 Hz with smaller peaks at 280, 1000, 1240, 1660 and 2200 Hz After subtraction of the background noise (thin line) the 620 Hz peak had the largest amplitude throughout the intensity range used in these experiment With the soft linings of the external auditory canal and the middle ear the attenuation of the higher frequencies is probably similar to those observed in the artificial ear employed Thus the frequencies above 1000 Hz have contributed relatively little to the results obtained particularly at the higher click intensities

The microphone output was calibrated in free field in a camera silentii A calibrated generator delivered a continuous tone of 1000 Hz When the tone had an intensity of 74 dB (re 0 0002 dyn/cm²) the microphone signal gave an output of 1 mV Other intensities were calculated by help of the following equation

$p = 74\ \text{dB} + \log \frac{p_2}{p_1}$ where p_1 is 1 mV and p_2 the peak

amplitude of the microphone signal in mV

The microphone had a linear characteristic for all frequencies of the click employed

RESULTS

Pattern and time course of MG inhibition

A conditioning click caused a long lasting inhibition of the response of MG cells to a subsequent test click

In Fig 2 A the upper trace shows the field potential of MG in response to a conditioning (*C*) and a test (*T*) click The responses consist of a negative wave (*N* wave) with superimposed spike followed by a positive wave (*P* wave) The second trace shows the click as recorded by the microphone The intensified portions of these two sweeps with the test response (*T*) are expanded and shown in the third and fourth trace In B C and D are shown similarly expanded responses to the

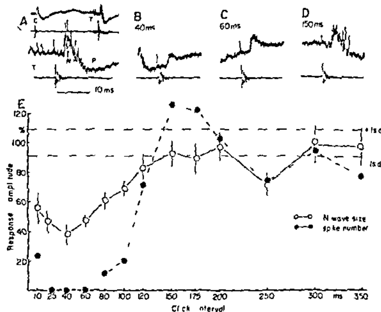


Fig 2 Time course of inhibition medial geniculate body (A) Rec medial geniculate neurones (upper) a click (120 dB) (second trace) tensified portions of these two tra expanded in the two lower traces As lower two traces in (A) b response was preceded by a con click (122 dB) at the indicated in The size of the medial genicula (O) and the number of spikes (● against the interval between the c ing and test clicks

same test click as they appear when preceded by a conditioning click at the indicated intervals The reduction consists of an abolition of discharges and a diminuition of the *N*-wave (B, C) On the other hand, at 150 ms interval (D) the *N*-wave was normal, but the number of spikes was larger than normal Such post-inhibitory rebound responses were often present, but varied in size and exact timing from one trial to the next The amplitude of such post-inhibitory rebound was, therefore, calculated from a number of trials *(E)* After the rebound, there was a new period with reduced responses giving a dip in the inhibitory curve at an interval of 250 ms

Two significant features of the MG inhibitory curve distinguishes it from the inhibition measured at the inferior collicular (IC) level First, the reduction of the *N*-wave of the medial geniculate was more pronounced than the effect on the corresponding *N*-wave of the inferior colliculus Second, the MG inhibitory curve shows a cyclic behaviour contrasting the single-peaked inhibitory curve from IC When measured as the probability of spike discharge the difference between the depression observed at the inferior collicular and medial geniculate levels became even more evident

Relative contribution of inhibition at inferior collicular and medial geniculate level

In order to obtain reliable results relative contribution to inhibition obta different relay stations, such compari made using results obtained from ind animals Unfortunately, the size of t potentials does not necessarily bear a relationship to the input sound pr However over a range from threshold saturation the inferior collicular and t *N*-waves and the cortical surface posit potential showed a remarkably simi velopment having near identical thr and saturation points The last point certain than the threshold value bec relatively large contribution of seve may contribute relatively little to th potential because of the increased dis of the synaptic potentials and unit dis which comprise the field potential In this restriction a relative indication contribution to the inhibition at vario tions was gained by referring the depre an input/output curve of the field pote that nucleus particularly when opera range given by the lower two-thirds

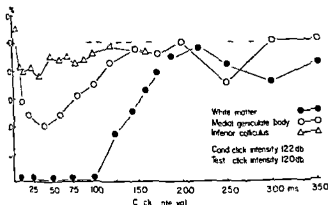


Fig 3 Effect of a conditioning click (122 dB) on subsequent responses to a test click (120 dB) recorded from the auditory radiation (positive killed end response ●) the medial geniculate body (N wave ○) and the inferior colliculus (*N* wave △) All responses are expressed as per cent of the control response (*ordinate*) in relation to the interval between the conditioning and test clicks (*abscissa*)

/e Since the active range of the sound sores that is from the threshold to the ration point was located at somewhat rent positions on the intensity scale in ous animals only the difference in dB was sured In this way each experiment ed as its own control

leasured as a percentage reduction of the onses relative to the control level the tive efficiency of the inhibitory processes various stations in the auditory system ared to differ Fig 3 gives the results from nes of experiments performed on the same A conditioning click with an intensity of dB produced a depression of the responses test click of 120 dB at both the inferior cular medial geniculate and auditory ical level The size of the *N* wave re led from the inferior collicular level is n as open triangles whereas the amplitude he medial geniculate *N* wave is given as n circles The symbols give the size of the response as a function of the interval from preceding conditioning click Both the IC the MG *N* waves are predominantly due he EPSPs spikes contributing only a small as seen during rapid tetanic stimulation he reduction of the *N* wave was larger in medial geniculate than in the inferior licular record However a significantly ater change occurred in the record from the te matter which indicates a reduction of the nber of discharging MG cells At intervals from 20 to 75 ms between the conditioning and test clicks there was total abolition of the discharge of MG cells After 200 ms the response was back to normal At longer intervals a new reduction of the discharge occurred indicating cyclic inhibition The cyclic type of inhibition was not seen below the MG level and corresponds to the pauses between the repetitive burst discharges of MG units in

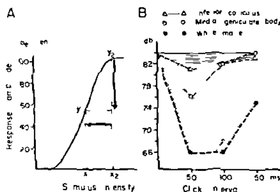


Fig 4 Relative contribution of various stations of the auditory nervous system to inhibition of click-evoked responses (A) Heavy line gives size of response (*ordinate*) as a function of stimulus intensity (*abscissa*) A response reduction from Y_2 to Y_1 (vertical arrow) corresponds to a reduction of the stimulus intensity from X_2 to X_1 (horizontal arrow) (B) Using the method sketched in (A) the inhibitory curves obtained from the inferior colliculus (△) the medial geniculate body (○) and the white matter (●) The inhibition produced by cells caudal to the inferior colliculus is represented by the horizontally striped area the inhibition added at the inferior collicular level by the obliquely hatched area and the inhibition added at the medial geniculate level by the dotted area

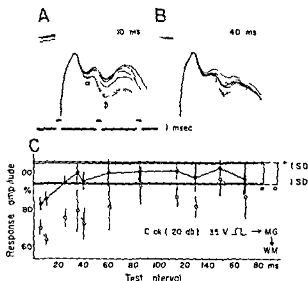


Fig 5 Test for postsynaptic excitability of MG neurones A The α deflection recorded from the auditory radiation indicates the number of postsynaptic elements excited directly by a stimulating micro-electrode in MG The β deflection indicates the number of cells excited transsynaptically by activation of intraneuclear presyn aptic fibres The stippled lines are tracings of super imposed control responses Superimposed on these rec ords are 5 other records (full lines) in which the test stimulus was preceded by a click of 120 dB at intervals of 10 ms (B) As (A) but with a click interval of 40 ms (C) Time course of inhibition of α (●) and β deflection (O) as a function of interval between conditioning (122 dB) and test stimulus of 35 V (*abscissa*) Vertical range bars give ±1 standard deviation

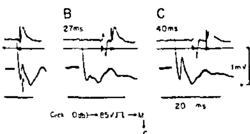
response to a click (Aitkin & Dunlop 1969 Etholm 1975)

Another way to measure the relative contri bution of the inhibition at various levels is to compare the inhibitory effect with the in put/output curve for each recording station obtained at the start of the experiment The relative reduction is calculated using the tech nique shown in Fig 4 A The input/output curve is drawn with a full line An uncon ditioned test stimulus of an intensity X_2 pro duced a response of amplitude Y_2 When preceded by a conditioning stimulus however the amplitude was reduced to Y_1 (vertical arrow) The observed inhibition is equivalent to a reduction of the input sound pressure from X_2 to X_1 (horizontal arrow) Using this tech nique to compare the inhibitory curves ob tained from the inferior collicular medial geniculate and white matter with the[...] tive input/output curves in a single ex[...] the graph in B was obtained Since the [...] size for its greatest part was depend[...] the size of the afferent volley the hor[...] hatched area mainly represents the i[...] generated below the inferior collicu[...] larly the obliquely hatched area re[...] size of the medial geniculate A wa[...] mostly is dependent upon the m[...] collicular fibres discharging There[...] short lasting inhibition seen here is a[...] of the contribution of inhibition at th[...] collicular level The large dotted a[...] ever was measured from the white m[...] indicates the reduction of medial g[...] neuronal discharge This area indica[...] fore, inhibition added at this level [...] none of these measurements are [...] lated to the inhibitory accretion [...] relative indication of the size of the [...] present

Postsynaptic inhibition

In accordance with the experiences [...] & Erulkar (1963) penetration of [...] geniculate neurones proved relatively [...] In some cells however it was p[...] record intracellularly for a short pe[...] membrane potential was always low [...] 40 mV) most likely due to damage [...] membrane The click was often c[...] eliciting a depolarizing wave (EPSP) [...] which there were one or two local [...] or abortive spikes followed by a [...] hyperpolarization (50–60 ms) interpr[...] IPSP because it corresponded to ce[...] spontaneous discharges Often this [...] was followed by a second IPSP a[...] single click was given

Another test for postsynaptic [...] can be made with the technique u[...] by Renshaw (1940) in the spinal [...] stimulating electrode inside the med[...] late body will excite postsynaptic el[...] well as presynaptic fibres Recorded [...] auditory radiation the excitation of [...]



Excitability tests of presynaptic fibres terminating med al geniculate body (A) Response of the inferior ulus (upper trace) in response to an 85 V pulse red through a tungsten micro-electrode located g the medial geniculate neurones The intensified n of the upper trace is expanded in the third trace n shows a negative spike signalling the antidromic on of inferior collicular neurones (arrow) (B) As but the test pulse was preceded by a click as ited by the microphone record (second trace) The al between the click and test pulse was 27 ms The om c spike was markedly reduced (C) As (B) but i click test stimulus interval of 40 ms The size of the omic field potential was increased above normal ling that the constant test pulse to the medial ulate body excited more fibres than in the control ion in (A) The increased antidromic field potential is asure of increased excitability of fibres of the num inferior colliculus terminating in the medial ulate body

iptic elements will be picked up as a short icy monophasic positive deflection This xemplified by the α deflection in Fig 5 A ilation of intranuclear presynaptic termi fibres will, after the synaptic delay also e discharge of the MG cells provided not ells are refractory following a too strong ct stimulation This will lead to a second ection in radiation record, labelled β

ease a click changes the excitability of the lear elements of the medial geniculate y a subsequent test stimulus will produce esponse which differs from the control ponse If the postsynaptic elements are erpolarized the direct stimulus will excite er neurones In the present experiments was indeed observed in the form of a uced α deflection (Fig 5 A) In addition β deflection was reduced (Fig 5 B) The ter observation could be due to either post aptic or presynaptic inhibition or to both e to a certain spontaneous excitability associated with the spontaneous barbiturate spindles, a series of records was measured at each interval in order to minimize any alterations other than those produced by the click itself In Fig 5 A and B are shown 5 superimposed control responses to stimulation within the medial geniculate body with a constant test stimulus (stippled lines) traced on top of each other Superimposed on these records are 5 records in which the test stimulus was preceded by a 120 dB click (full lines), the interval being 10 ms in A and 40 ms in B There was a small, but significant de crease of the α deflection when the click interval was shorter than 30 ms A somewhat greater reduction occurred in the β deflection However, this deflection may be due to either postsynaptic or presynaptic inhibitory mecha nisms or to a combination of the two When the click test interval was increased to 40 ms, no definite reduction of the α deflection could be measured, whereas a small reduction of the β deflection was still present The time courses of the reduction of the two deflections are seen in C As expected from the situation in other thalamic nuclei the duration of the α wave depression was considerably shorter than that of the β deflection (Andersen et al , 1964) This indicates that the synaptic excita tion is a more sensitive test for cell excitability than the directly stimulating electrical current

The relative depression of the α and β deflections was dependent upon the test strength If the latter was too strong, no de pression was seen at all in the α deflection, possibly due to current spread to the neigh bouring Ranvier nodes, thus escaping the hyperpolarization which most probably took place at the cell body (Maekawa & Purpura, 1967) The time course of the depression of the deflection roughly coincided with the depres sion of spike discharge and reduction of the N-wave

Similar reductions in the α and β deflection were also seen when the test shock was delivered in the trough of spontaneous

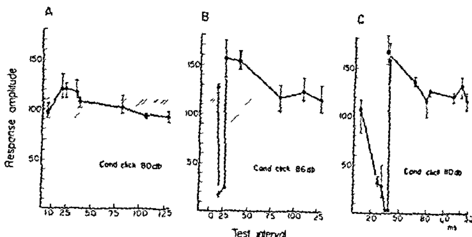


Fig 7 Time course of the excitability changes in fibres from the inferior colliculus terminating in the medial geniculate body (A) A conditioning click of 80 dB caused a weak increase of afferent fibre excitability measured as size of antidromic spike recorded from IC (B) More pronounced effect by a 86 dB conditioning click except at short intervals where there was a sudden reduc antidromic field potential signalling blocked in large number of cells (C) A conditioning click gave an even more pronounced initial re long-lasting increase of brachium fibre excitab

spindle activity of the MG cells In such cases, however, the degree of the inhibition was smaller than that following a strong click

In conclusion, testing the postsynaptic excitability of MG cells with the Renshaw technique (1940) gave evidence that part of the inhibition observed in the medial geniculate body involves postsynaptic inhibition

Presynaptic inhibition

In order to test for presynaptic inhibition, a modification of the original Wall (1958) technique was used A major part of the orthodromic afferent impulses to the medial geniculate body comes from the ipsilateral inferior colliculus Thus by stimulation within the medial geniculate body terminal fibres of the brachium of the inferior colliculus should be excited, giving rise to antidromic invasion of inferior collicular neurones This was found to be the case In Fig 6 the upper trace shows a record of a micro electrode in the inferior colliculus and the second trace gives the click response The intensified part of the inferior collicular response is expanded and shown in the lower trace Following the stimulus artefact, there was a short negative deflection with a latency of about 0 9 ms (Fig 6A, arrow) The amplitude of the deflection was in the centre of the inferior collicular and fell quickly outside its border F more, with high impedance electro deflection was seen to be composed of discharges having short and constant la

Therefore, this deflection is interp due to antidromic invasion of a num inferior collicular cells

Because a series of linearly inc stimuli delivered through the medial ge stimulating electrode gave gradually in ing antidromic responses, the size antidromic inferior collicular respon taken as an indicator of the number brachium inferior collicular terminal which was excited

A conditioning click produced a la sponse in the inferior colliculus (Fig upper trace) In the test response, deliv ms later there was a near total reduc the antidromic response However whe click test interval was increased to 40 m the antidromic test potential was inc above the control record implying a larger number of presynaptic terminals i medial geniculate body had been stimula

The time course of the excitability ch

ıree different strengths of conditioning s are given in Fig 7 With relatively weak tioning clicks (80 dB) the excitability of brachium inferior collicular terminals in MG showed an increase with a maximum cl ck test interval around 25 ms The t was relatively weak and lasted only 0 ms On increasing the conditioning click) dB however the picture changed The tab l ty rise was more prominent showing aximal amplitude increase of more than and a total duration of more than 100 ms ever at intervals between 20 to 27 ms was a nearly complete abolition of the dromic test response This marked reduc of the antidromic response at intervals nd 25 ms was a constant feature with all ng conditioning clicks With a 110 dB i t oning click for example the duration he block increased somewhat so that it d until 35 ms After that time however ant dromic response was markedly en ed again indicating an increase of the tability of the fibres terminating in the lial geniculate body

DISCUSSION

k parameters

excitability tests like those used in the sent investigation it is important that the nd stimulus was delivered as abruptly as s ble The time course of the click used in present study (Fig 1 A) fulfils this crite 71 The initial deflection started suddenly) much larger than the subsequent deflec ts and was part of the main frequency ing a peak at 620 Hz With increasing ngth of the clicks the amplitude above kground noise of the major frequency nponents increased l nearly At the highest k intensities used there was a small reduc n of the amplitude of two of the highest quency peaks 1240 and 2200 Hz Thus th increasing click strength there was no crease of any part cular frequency which uld account for a systematically different conditioning situation than that used at lower stimulus strength The low background noise (around 30 dB) at the main frequency of 620 Hz was acceptable for the tests used in the present investigation

Duration and relative size of inhibition at inferior collicular geniculate and cortical level

A main feature of the inhibitory curves obtained at the various stations of the auditory system was their large difference in duration At the inferior collicular level the maximal inhibition did not last beyond 50 ms whereas the inhibition at the geniculate and cortical level lasted for about 120–150 ms Further more at the two last levels the inhibition was clearly cyclic corresponding to the repeated *P* waves that are seen in response to a single click at appropriate levels of barbiturate anaesthesia A probable explanation is that the medial geniculate body contains an inhibitory mechanism that is different from that operat ing at lower levels of the auditory system

A second feature of the inhibition found at various aud tory stations is its effectiveness or size In order to measure the relative size of the inhibition at the d fferent levels it was necessary to measure this against an in put/output curve for each recording station A prerequisite for using the input/output curves to measure the relative inhibition at various auditory stations is that the threshold is the same that the increase shows a similar pattern at all levels to be compared From the thresh old to 20–25 dB above threshold this was found to be the case Within this range of the input/output curves it was obvious that the inhibition occurring at the medial geniculate level added greatly to the tot il inhibition in the auditory system

The MG inh bition is probably of the sur round type being largely responsible for the very n irrow tuning curves obta ned it the medi il genicul ite level (Katsuki et al 1959*a*) The cyclic nature of the inhibition is exem plified by the post stimulus h stogra

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B Etholm M D
Vestfold Sentralsykehus
Øre–Nese–Halsavdeling
N 3100 Tønsberg
Norway

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COCHLEAR INNERVATION IN THE GUINEA PIG

II *The Spiral Tunnel Bundle*

Ch G Wright and R E Preston

From the Kresge Hearing Research Institute, University of Michigan Medical School Ann Arbor Mich 48104 USA

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·t The organization of nerve fibers in the spiral bundle (STB) of the guinea pig organ of Corti has tudied by light and electron microscopy In the ırn STB fibers were found to run toward the base or about equal numbers while in the upper turns they ɛdominantly in an apical direction Long spiral 's of 100 microns or more in length were abundant hird and fourth turns but were rarely seen in the ırns No synaptic contacts between tunnel bundle ould be found and no afferent nerve fibers were ı the bundle Furthermore interruption of the t nerve supply resulted in complete degeneration of 3 The tunnel bundle does not therefore appear to te of synaptic interaction between afferent and t neural elements

ɛ basis of electron microscopic observa ɔf material from the cat, Spoendlin (1969) ɔncluded that over 90% of all cochlear nt nerve fibers terminate on the inner ells In addition, a recent quantitative ıs of the nerve supply of the guinea pig ɛa by Morrison et al (1975) provided nce that 85–90% of the afferent fibers ɱ inner hair cells Spoendlin's work on ferent innervation of the cat has stimu increasing interest in the role of neural 'nts near the inner hair cells in informa- processing at the periphery (Lynn & rs, 1970 Nieder, 1971, Zwislocki & bch 1973)

e idea that interaction between the vari ypes of nerve fibers in the vicinity of the inner hair cells is possible and of significance in determining the final electrical output of the cochlear receptor is supported by anatomical studies demonstrating synaptic contacts between these fibers Smith (1968) and Spoendlin (1966) have, for example, found synapses between efferent and afferent fibers in the region of the inner spiral bundle (ISB) In addition to the synapses made by the ISB fibers, Engstrom & Engstrom (1972) have reported that spiral tunnel bundle (STB) fibers form synaptic contacts with afferent nerve fibers in the guinea pig

As part of a survey of neural organization in the region of the inner hair cells of the guinea pig, we have made a detailed study of the spiral tunnel bundle using both light and electron microscopy In this investigation an effort has been made to gather additional data which may aid in assessing the significance of the STB for information processing inside the organ of Corti

METHODS

The present study represents an extension of our work on the inner spiral bundle (Wright, 1975) Light microscopic observations of the STB have been made on silver-stained, whole mount preparations from the 14 experimental animals used in the ISB study These animals received lateral brain stem lesions designed to interrupt the efferent nerve

ʺt for this study was provided by the Deafness rch Foundation NINDS USPHS Research Grant ·065 and Program Project Grant NS-05785

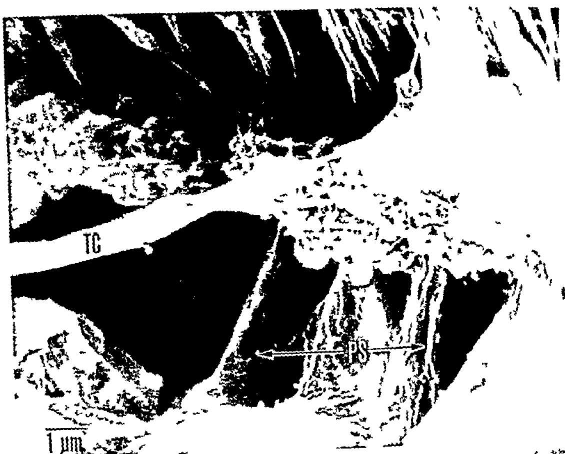


Fig 1 Scanning electron micrograph of the spiral tunnel bundle (*STB*) in the second turn of a normal guinea pig cochlea *TC* upper tunnel-crossing nerve fa... the STB *PS* stalks of inner pillar cells

supply of the inner ear on one side The postoperative survival times were from one to three weeks in most cases A detailed description of the lesions survival times and staining technique is found in our previous report In addition to the preparations from experimental animals the innervation was studied in whole mounts from a large collection of normal material

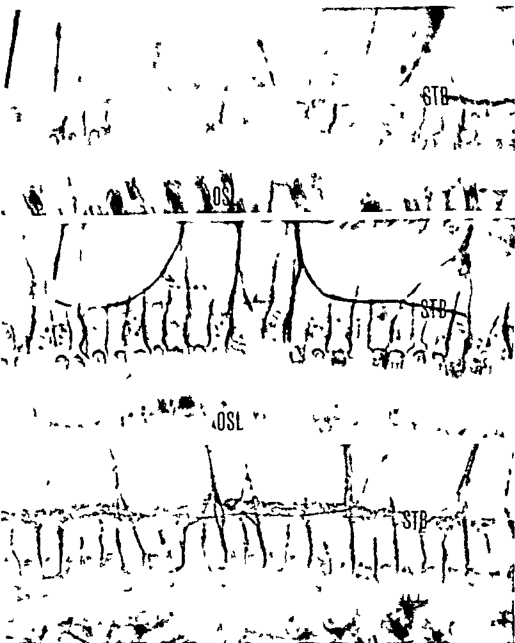
Altogether 14 animals were used for electron microscopic study Five of these had successful lateral brain stem lesions with survival times of 18 hours 24 hours 2 days 5 days and 28 days The remaining animals were untreated controls The methods used for preparing tissue for both scanning and transmission electron microscopy are given in an earlier publication (Wright & Preston 1973)

RESULTS

A scanning electron micrograph of the ... tunnel bundle is shown in Fig 1 ... the normal position of the bundle in the ... turn of the guinea pig cochlea The STB ... just inside the tunnel of Corti ... stalks of the inner pillar cells near the ... the tunnel space In this micrograph a ... tunnel-crossing fascicle and numerous ... ings on the individual fibers of the bundle c... easily be seen

Light microscopic observations

Light micrographs of silver stained ... parations showing the tunnel bundle a... upper middle, and basal portions of the ... of Corti can be seen in Fig 2 With the ... stain used in this study there was also ... striking variation in the degree of ...



t micrographs of silver stained whole mount from normal guinea pigs showing the spiral le (*STB*) in the upper third (*A*) second (*B*) and mid-basal (*C*) cochlear turns The bars at lower left represent 10 μm in each case *OSL* osseous spiral lamina *ISB* region of inner spiral bundle

n of different components of the In all turns of the organ of Corti ained nerve fascicles were present ood out sharply against a diffuse background of lightly stained ele he darkly stained fibers were always more numerous in the third and apical turns of the cochlea In the lower turns only scattered short fascicles were heavily impregnated in the more diffusely stained substance of the bundle The heavily stained fascicles consistently longer in the upper p

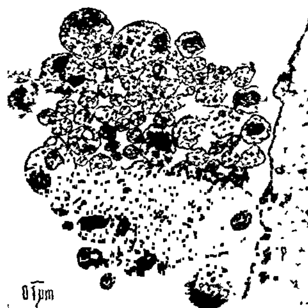


Fig 3 Transmission electron micrograph showing a cross section of the spiral tunnel bundle in the middle third turn of a normal guinea pig cochlea In this specimen approximately 90 fibers are present in the STB *IP*, inner pillar cell

cochlea than in the lower turns where the fibers usually ran for no more than 50 µm in the STB before they left the bundle to cross the tunnel space The long fascicles in the third and fourth turns were difficult to measure accurately since their points of entry into and exit from the bundle were often impossible to locate However, some of these fascicles could be traced for 250 to 300 µm before they were lost and it was apparent that longer ones were present, particularly in the apical turn. Indeed, in a preparation from the fourth turn in which most of the STB fibers had degenerated as a result of a brain stem lesion it was possible to trace a tiny fascicle (probably composed of a single fiber) over a distance of about 600 µm toward the apex Short fibers running for a few microns up to 40 or 50 µm were present in all turns including the third and fourth where they were intermingled with long fascicles Toward the lower basal part of the organ of Corti, a few darkly-stained fibers were seen in the bundle and those that were found usually had a course of less than 20 microns in the STB It appeared that, in parallel with the inner spiral bundle (Wright, 1975), the overall size of the tunnel bun[...] reduced in the hook portion of the [...] In fact, by light microscopy the [...] seldom discernible at all in [...] hook.

Darkly-stained fascicles were [...] the tunnel bundle from the ISB [...] toward either the base or the a[...] cochlea before leaving the STB t[...] tunnel However, in the second [...] fourth turns fibers coursing towa[...] definitely predominated STB fi[...] basal turn appeared to run toward[...] base or apex in about equal nu[...] example, of 28 shorter fibers (less t[...] in length) measured in the ap[...] normal ears, 20 ran toward the [...] toward the base. In a sample of 67 [...] the third turn, 48 were found to co[...] the apex; 19 ran toward the base O[...] turn fibers 30 coursed in an apic[...] and 12 ran toward the base Fi[...] fibers in the basal turn 19 ran towa[...] while 16 coursed in the direction [...]

Almost all fibers that left the tu[...] crossed the tunnel of Corti to the [...] cells However, a few were fou[...] entered the inner spiral bundle T[...] entered the STB from the inner s[...] and after a short course in the STB [...] the ISB Such fibers were rarely see[...] found only in the upper turns Also[...] and fourth turns, fibers were occasi[...] to leave the STB, run longitud[...] tunnel space, and then return to [...] bundle In their longitudinal course[...]

Table I

Cochlear turn	Ear			
	A*	B*	C	[illegible]
Apical	30	40	32	[illegible]
Third	45	40	90ᵇ	[illegible]
Second	95	110	90	[illegible]
Basal	145	135	–	
Lower Basal	60	–	69	

* Ears from same animal ᵇ Shown in Fig 3



Long tud nal sect on of the spiral tunnel bundle how ng a radial fasc cle of f bers pass ng between er p llar cells (*IP*) and entering the bundle The arrows ind cate two large swell ngs on tunnel bundle f bers wh ch contain dense-cored ves cles

ually crossed over several radial tunnel les A small swelling was often found on ongitudinal fiber at the point where it d a radial one Longitudinally oriented were seen rather frequently in the upper and many of these appeared to be looping rom the STB rather than crossing the l in an oblique course However oblique l crossers were also noted occasionally

ron microscopic observations

ossible to count accurately the number of s present in the STB only in cross sections e bundle examined by electron micro y An example of one such cross section particularly large tunnel bundle is shown g 3 Fiber counts were made using tissue ples from five cochleas taken from four nal guinea pigs Table I shows the number bers found in the various turns of the organ orti in these ears It will be noted that there was considerable variation in the size of the tunnel bundle between different animals The STB was consistently largest in the mid basal turn and the number of fibers in the bundle was reduced at both the apical and basal ends of the cochlea

Electron microscopy was attempted on some of the material fixed in 10% formalin and stained with silver As expected this tissue was not adequately preserved for detailed electron microscopic study However it was obvious that some fibers apparently scattered at random throughout the bundle were encrusted with a particularly dense granular precipitate These fibers apparently correspond to the darkly stained elements observed by light microscopy With the electron microscope no evidence was found to indicate that fibers of a particular type or in any specific position within the bundle were more likely to be heavily stained

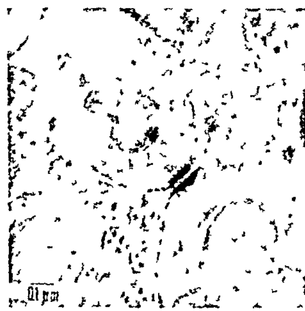


Fig 5 Desmosomal contact between fibers of the spiral tunnel bundle in the mid-basal turn

The large swellings frequently found on tunnel bundle fibers are filled with mito chondria and vesicles like those usually as sociated with synapses We have noted in agreement with Engstrom & Engstrom (1972) that some fibers also contain large dense cored vesicles as seen in Fig 4 which shows a longitudinal section of the tunnel bundle Us ing material fixed in either glutaraldehyde or osmium tetroxide we have made a careful search by electron microscopy for synaptic contacts in the tunnel bundle Both cross sections and longitudinal sections up to 100 µm in length were studied in cochlear tissue from normal animals and from experimental animals with brain stem lesions On rare occasions symmetrical membrane thickenings characteristic of desmosomes were found and an example of one such structure is shown in Fig 5 However we were unable to find any evidence of contacts which met the established morphological criteria for synapses in the spiral tunnel bundle of the guinea pig It is also of interest to note that fibers having the morphological characteristics of cochlear afferents were never seen in or passing through the tunnel bundle

Brain stem lesions

The degeneration of the inner spiral bundle following brain stem lesions designed to int rupt the efferent nerve supply to one ear described in a preceding paper (Wright 197 It was found that the number of tunnel bundle fibers that degenerated as a result of thes lesions very closely paralleled the degr of degeneration in the ISB That is in th animals in which there was complete degener ation of the ISB there was also a total dis appearance of STB fibers Complete degener tion of both bundles occurred in those ani in which the lesions had a more medial pos tion (Wright 1975 Fig 1 and related discu sion) Some fibers remained in both t inner spiral and tunnel bundles in the anim that received the more laterally placed lesion

In addition to the experimental mat studied with the light microscope electr microscopic observations were made on coc lear tissue from 5 animals with survival tim of 18 hours 24 hours 2 days 5 days and days Little evidence of degeneration was se in the tunnel bundle in the animals with 18 a 24 hour survival times although degener efferent terminals were noted beneath t outer hair cells Extensive degeneration tunnel bundle fibers was found in the ani having a 2-day survival time but some norm appearing fibers were still present In animals with survival times of 5 days and days no normal STB fibers were found in a turn of the organ of Corti

DISCUSSION

In this investigation light microscopic obser tions on the organization of the tunnel bun have been combined with electron mic scopic study of its ultrastructure in an effort obtain a more complete picture of this port of the cochlear nerve supply

Electron microscopy done on silver stain preparations indicated that darkly stained fi ers seen by light microscopy represen random sample of the STB fiber population

true, it can be concluded that the fibers upper turns tend to run predominantly in ical direction Many of these fibers run ng distances (200 μm or more) in the On the other hand, tunnel bundle in the basal turn of the guinea pig a were found to be uniformly short and n toward either the base or apex in equal numbers

fact that a larger proportion of the STB is consistently stained darkly in the third and apical turns may account for ndez' (1951) observation that there was rease in size of the tunnel bundle toward ex in some of his silver preparations ounts of tunnel bundle fibers, based on n microscopy, are in reasonable agree with those of Engstrom et al (1966) for inea pig However, we did find a consid- variation between animals in size of the The tunnel bundle was found to be t in the middle to upper basal turn with decreases in size at both the apical and ends of the cochlea

he apical turn we were able to trace some mall fascicles for distances of 250 to 300 Many of these showed no divisions or able decrease in size within the tunnel e suggesting that at least some of their tuent fibers extend the entire length of scicle These measurements agree with ndez (1951) finding that some fibers e 300 μm in the STB It was apparent in reparations that some fibers have longer es in the bundle but they generally not be accurately measured However ny fascicle was followed over 600 μm in enerating tunnel bundle

rnandez (1951) states that all STB fibers tually turn out to the outer hair cells In a nt study of zinc iodide-osmic acid-stained rial (Wright & Preston 1973) we found some tunnel bundle fibers returned to the r spiral bundle after running for a short nce in the STB Such fibers have rarely observed in our silver stained pre- tions However a few fibers were seen to turn back into the ISB from the tunnel bundle in the third and fourth turns

Engstrom et al (1966) noted fine fibers running in a longitudinal fashion in the tunnel of Corti in the guinea pig Such fibers were also visible occasionally in our silver stained material Many of these could be seen to originate from the tunnel bundle, loop out into the tunnel space, and then return to the STB Other longitudinal fibers appeared to be cros- sing the tunnel to reach the outer hair cells via a long, oblique course

Numerous enlargements or swellings are present on the fibers of the tunnel bundle These are filled with mitochondria and vesi cles which suggest the existence of synapses In fact, Engstrom & Engstrom (1972) have reported that they found synaptic contacts in the STB of the guinea pig We were therefore surprised that an extensive study of the tunnel bundle by electron microscopy revealed no evidence of synaptic profiles However, sym metrical membrane thickenings such as shown in Fig 5 were seen on a few occasions We are inclined to agree with Spoendlin (1966), that these structures represent desmosomes rather than functional synaptic contacts In addition, we found that all fibers of the tunnel bundle degenerate after appropriately placed lesions are made in the brain stem This result is in agreement with the finding of Smith & Rasmussen (1963) in the chinchilla Spoendlin (1966) in the cat, and Morrison et al (1975) in the guinea pig Also we were never able to locate afferent fibers within or passing through the STB These observations indicate that the spiral tunnel bundle is composed entirely of fibers of the efferent type There would thus appear to be little opportunity for synaptic communications between efferent and afferent nerve fibers in the STB How- ever this does not rule out the possibility that efferent fibers of different types may occa sionally synpase with one another within the bundle Engstrom & Engstrom (1972) have described fibers in the ISB and STB which contain dense-cored vesicles and appear to

represent a specific group of efferent fibers We have observed such fibers in the tunnel bundle in this study Their exact origin and nature is, however, still unclear In a recent investigation utilizing the squirrel monkey, Nakai & Igarashi (1974) found the tunnel bundle to be composed of fibers from both the crossed and uncrossed portions of the efferent nerve supply It might be noted that preliminary experiments in our laboratory indicate that this is also the case in the guinea pig

It is of interest to recall that the STB is not a constant feature of cochlear innervation among the mammals The bundle is present, for example, in some species of rodents, such as the guinea pig and chinchilla, and absent in others such as the rat (Smith & Haglan, 1973) It may be that in those animals in which the tunnel bundle is present it simply represents an outlying portion of the inner spiral bundle made up of efferent fibers destined to innervate the outer hair cells Any significant synaptic interaction between afferent and efferent neural elements is probably confined to an area closer to the habenula perforata within the inner spiral bundle proper

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ZUSAMMENFASSUNG

Die Organisation der Nervenfasern im spiralen Tunnelbundel (STB) des Cortische Organs von Meerschweinchen wurde licht und elektronenmikroskopisch untersucht In der basalen Win ... verliefen die STB Fasern in etwa gleicher Anzahl ... Basis wie zur Apex wahrend sie in den oberen Windu... en vorwiegend apikal verliefen Lange Spiralfasern ... M kron oder langer waren haufig in der 3 und 4 Wind... aber selten in den unteren Windungen Zwischen den Fasern des Tunnelbundels konnten keine synaptischen Kontakte ge... werden und ebenso wurden keine afferenten ... Fasern im Spiralb...

sein

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C G Wright Ph D
Kresge Hearing Research Institute
University of Michigan Medical School
Ann Arbor Mich 48104 USA

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INNER EAR DAMAGE AND HEARING LOSS AFTER EXPOSURE TO TONES OF HIGH INTENSITY

T R Dolan,[1] H W Ades, G Bredberg* and W D Neff

From the Bioacoustics Research Laboratory University of Illinois Urbana Ill and the Center for Neural Sciences Indiana University Bloomington Ind USA

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r Experimental animals (cats) were exposed to f 125 1000 2000 and 4000 Hz at sound pressure n the range 120 to 157 5 dB and for durations of ur (1000 2000 4000 Hz) or four hours (125 Hz) ne audiograms were obtained for each animal be d after exposure Post-exposure tests were con until complete recovery of hearing had occurred or i stable permanent threshold shift had been ed Cochleas of animals were examined by ontrast microscopy condition of all hair cells was d Extent of inner-ear damage and range of fre es for which hearing loss occurred increased as re tone was decreased in frequency For example re to 4000 Hz produced damage in a restricted of the cochlea and hearing loss for a relatively range of frequencies exposure to 125 Hz pro wide spread inner ear damage and hearing loss hout the frequency range 125 to 6000 Hz

the early days of experimental studies of ng investigators have used tones or noise gh intensity to stimulate the ear of an rimental animal and thus, produce dam o structures and change in function of the ear In a review of the litterature on the lem of stimulation deafness , Kemp) cited 44 published reports of clinical and experimental investigations The earliest clini cal study mentioned that went beyond casual observations was published in 1890 (Haber mann), the earliest experimental study, in 1907 (Wittmaack)

With the development of new techniques of examining inner ear pathology and of measuring inner ear function, important new informa tion has come repeatedly through the use of this old procedure which allows physical characteristics of the exposure stimulus to be correlated with inner ear pathology, stimulus characteristics, with changes in inner ear func tion, and inner ear pathology, with changes in inner ear function

Major advances in methods of detecting and mapping inner ear damage have included (in more or less chronological order) 1) improved techniques of sectioning and staining of mammalian cochleas, 2) graphic reconstruction of cochleas from serial sections, 3) surface preparation of organ of Corti and use of phase-contrast microscopy to examine and map all hair cells, 4) examination of selected regions of organ of Corti by transmission electron microscopy, 5) examination of cochlear structures by scanning electron microscopy

The important methodological advances in studying inner ear function have been 1) re cording electrical responses produced by inner ear elements, 2) use of behavioral meth

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[1] at Parmly Hearing Institute Loyola University of go Chicago Illinois

* at Department of Otolaryngology University of ala Sweden

obtain accurate measures of auditory discrimination (absolute and differential thresholds)

In the majority of published reports, inner ear damage has been assessed and related to frequency, intensity, duration and complexity of the sounds used to produce damage, or change in inner ear function as measured by electrophysiological or behavioral techniques has been related to physical aspects of the exposure stimulus There have been a relatively small number of studies in which inner ear damage and inner ear function have been examined in the same animal after controlled exposure to sound stimuli *In the earliest of* these experiments, inner ear pathology was assessed by examination of selected serial sections through the cochlea, graphic reconstructions were made according to the method of Guild

Bredberg & Hunter Duvar (in press) have recently published a summary of the results of *animal experiments and human studies in* which changes in hearing have been related to inner ear damage that was produced by exposure to high intensity sounds or by other means, such as surgery or administration of ototoxic drugs

Through the use of the surface preparation of the cochlea and examination by phase contrast microscopy a more precise and complete picture of damage to the organ of Corti can be obtained In a preliminary report, Dolan et al (1970) described the results of an experiment in which the hearing of cats was tested before and after exposure to pure tones at high sound pressure levels After sacrifice, surface preparations of the cochleas were made and all hair cells and adjacent structures were examined by phase contrast microscopy Details of this study including results not available at the time of the preliminary report are given below

METHODS AND PROCEDURE

The general scheme of the *investigation* included pre-exposure audiometric testing controlled exposure to a potentially destru stimulus, post exposure audiometric tes and post-mortem examination of the inner

Prior to initial training of the cats one c lea of each animal was destroyed by surg Additionally, the pinna of the experiment was removed and the surrounding area reconstructed Removal of the pinna advantageous in that it decreased the var in intensity of acoustic stimuli at the ear due to positional changes of the pinna d behavioral testing The absence of a pinna allowed close examination of the ear prior to and following exposure acc monitoring of acoustic wave-forms durin posure, and easy accessibility for cleani the external canal

Exposure

For exposure to a high intensity sound (t each experimental animal was anesthe with diabutal and positioned in a stere instrument Its head was held only by the bar

gener

oscillator The output of the oscillator wa through an Altec 1569 A amplifier and in Altec 802 D speaker The *output o* speaker passed through an adapter cons of telescoping brass tubes (this allowe tuning by varying the length of the tube) finally into a speculum that was inserted the pinnectomized ear The sound inte was monitored through a *1 mm probe* placed just beyond the orifice of the specu the probe was connected to a *1/2 inch*

power supply) and fed *into* a Genera sound vibration analyzer Sound and d tion data were calibrated with a B&K pistonphone and probe calibration couple

For the data presented in the *Results* tion, frequencies of 125 Hz *1000 Hz* Hz, and 4000 Hz were *examined* At

ency, the effects of sound pressure levels also investigated

ioral testing

grams were obtained by an avoidance tioning procedure In order to avoid , the experimental animal was trained to from one side to the other of a double cage when a series of tone pulses was nted Each tone pulse had a duration of econd and rise and fall times of 300 msec The interval between pulses was one-econd On any given trial, the tone pulses presented for a period of ten seconds ved by shock given through the bars that d the floor, sides, and top of the double-age

ne pulses were generated by a General) 1310-A oscillator, amplified by a tosh MC-250 amplifier, gated without re-to phase by an electronic switch (Olsen udwig, 1965), and presented via an Lansing 802 D speaker The loud er and double grill cage were located in a l-deadened, double-walled room Sound ting and control instruments were out-of the room The experimenter sat at a ol panel and could observe the experi al animal through a one-way window

ter an animal had learned to make avoid-responses to tones well above threshold , the minimum sound pressure level (SPL) hich the animal would respond was de-ined at each of the following frequencies 250 500, 1000, 2000, 4000, 8000, and 0 Hz The general procedure for de-ining a "threshold" for a given frequency lved the attenuation of the tone by 15 dB each correct response When a level was hed at which no response was made, the was increased by 15 dB and then at ated in 5 dB steps until another failure irred This procedure was repeated until animal reversed his behavior from "re-nding to "not responding" in the same 5 step twice in succession Following de-ination of a "threshold" in this manner, the tone was increased by 50 to 80 dB and the entire procedure repeated Two "thresholds" were obtained at a single frequency during each test session Pre exposure testing was continued until stable audiograms had been obtained, that is, until the range of threshold values at each frequency had decreased to 10 dB or less for several successive tests

A similar procedure was followed after ex-posure to a high intensity tone Audiograms were measured until recovery to normal pre-exposure levels occurred or until the amount of measured threshold shift remained constant for a period of several weeks The time from exposure until sacrifice varied from two to four months for the animals included in the present report

Post-mortem anatomical methods

The preparation of the cochleas for post-mortem examination was essentially similar to that described in detail by Engström et al (1966), except that the entire organ of Corti was displayed on slides as surface specimens All hair cells were counted and the results recorded on punch cards for computer analysis The resultant cochleograms give a picture of the remaining or intact hair cells

RESULTS

The behavioral data showing amount of hear-ing loss (PTS, permanent threshold shift) and the anatomical data showing the percent of hair cells missing as a result of the exposure to a tone of high intensity are summarized below for 16 animals The amount of hearing loss suffered by an animal at a particular frequency is the difference in the median value of the final six measures obtained prior to the expo sure and the final six measures prior to post-mortem examination

Exposure to 4000 Hz

The behavioral and anatomical results for three animals exposed to a 4000 Hz tone are

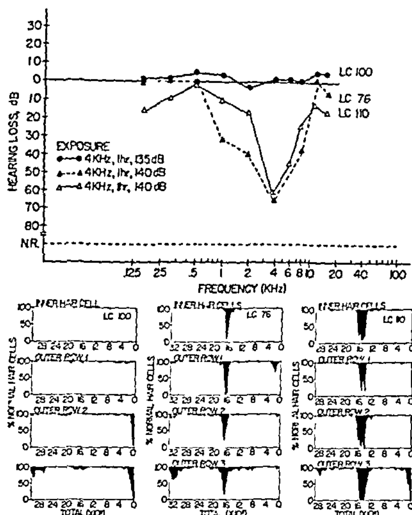
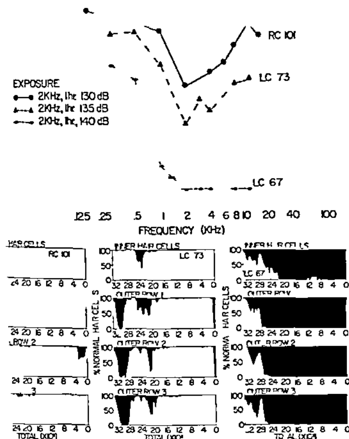


Fig 1 Pure tone thresholds (upper half of figure) and plots of inner ear damage (lower half of figure) for animals exposed to 4000 Hz tone For each animal, the O–dB line s its preoperative hearing level Hearing loss or permanent threshold shift (PTS) is plotted as a deviation from the O line In the plots of inner ear damage the base line (labelled 0, 4 8 12 etc) indicates the number of hair cells as counted starting at the base of the cochlea For example, 4 means that to that point four hundred hair cells had been counted The number of destroyed (aged hair cells is shown by the black shaded are example, in the plot for Cat LC 110, all of the hair outer row 3 were damaged in the region occupied cells 1400 to about 1600 (upper basal turn) The (was less in the other two rows of outer hair cells an single row of inner hair cells NR=No response to t maximum level available

shown in Fig 1 The top half of Fig 1 shows the amount of hearing loss (relative to preexposure audiogram) as a function of frequency The boxes in the lower half of Fig 1 show the percentage of normal hair cells remaining after the exposure as a function of the total cumulative number of hair cells measured from the basal end of the cochlea Region and extent of absent or damaged hair cells are indicated by blackened areas All three animals receive exposure of one hour duration Cat LC was exposed to a 135 dB SPL tone,[1] cats L and LC 110, to a 140 dB SPL tone

As indicated in Fig 1, cat LC 100 suff no hearing loss as a result of the expos Cats LC 76 and LC 110 had hearing losse the frequency range 500 to 12000 Hz maximum loss occurring at the exposure quency At 4000 Hz there was a perma hearing loss of approximately 60 dB

[1] All sound pressure levels are expressed in dB re 0 00002 pascal



re tone thresholds and inner ear damage of ani
J to 2000 Hz (F g 1 for explanat on)

l also shows that cat LC 100 sustained ly no hair cell destruction as a func the exposure Only slight damage is along the outer (3rd) row of outer ls (OHCs) Cats LC 76 and LC 110 had but narrow lesions in all three rows of and in the single row of inner hair cells lesions occurred in the upper basal turn midway along the length of the cochlea

ire to 2000 H.

sults of exposure to a 2000 Hz tone that ne hour in duration are shown for three ls in Fig 2

RC 101 was exposed to a 130 dB SPL it sustained a hearing loss in the range to 12000 Hz with a maximum loss of about 38 dB occurring at the frequency of the exposure tone No hearing loss was found for either the very low or very high frequencies Cat LC 73 exposed to a 135 dB SPL tone had greater hearing loss than RC 101 for all frequencies from 500 Hz to 12000 Hz Again the maximum loss about 58 dB occurred at the frequency of the exposure tone Cat LC 67 was exposed to a 140 dB SPL tone it had complete loss of hearing for frequencies from 2000 to 12000 Hz severe loss at 1000 Hz and smaller losses at 500 and 250 Hz

The anatomical results of the exposures to a 2 kHz tone are given in the lower half of Fig 2 Cat RC 101 which had a 38 dB hearing loss at 2000 Hz had only minor hair cell damage With the exception of a very narrow lesion in

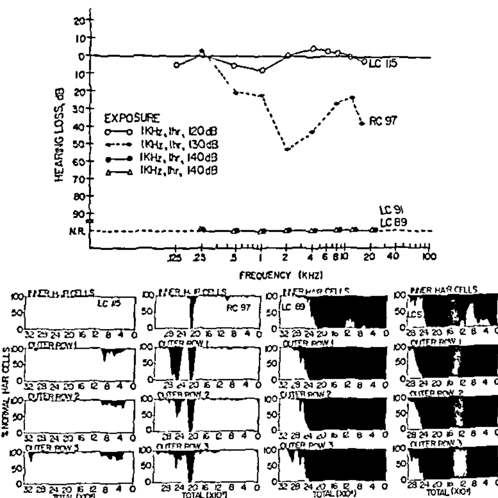


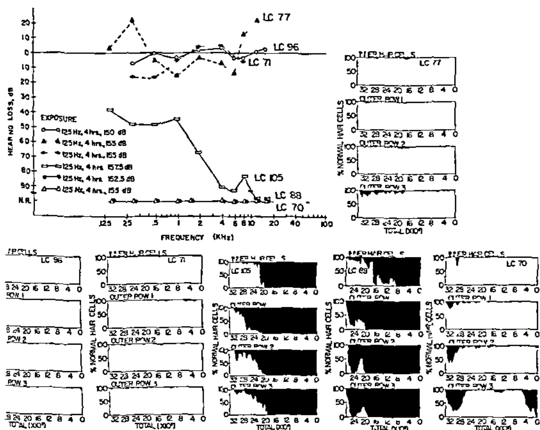
Fig 3 Pure tone thresholds and inner ear damage for animals exposed to 1000 Hz (Fig 1 for explanation)

which approximately 40% of the OHCs in the first row were missing, cat RC 101 appeared to have a nearly normal cochlea Cat LC 73, which had a 58 dB hearing loss at 2000 Hz, had severe destruction of both inner and outer hair cells, the greatest damage to the outer hair cells occurred in two regions toward the apical end of the cochlea One region of severe damage was in the approximate location of maximal activity caused by a 2000 Hz tone, the second region was at the apical tip of the cochlea and is apparently not reflected in the audiometric data It should be noted that the lesion that occurred more distant from the apical end is much like the lesions found in cats LC 76 and LC 110 as a result of exposures to a 4 kHz tone, the lesion is confined to a very limited region of the basilar membrane

The anatomical results of cat LC 67 suffered the greatest PTS as a result 2000 Hz exposure, are also shown in As expected from the audiometric d LC 67 had complete destruction of IH of all three rows of OHCs in all but th tip of the cochlea

Exposure to 1000 Hz

The audiometric results for four anim posed to a 1 kHz tone for one hour are in Fig 3 Exposure levels were 120, 1 140 dB Cat LC 115 was exposed to a SPL tone and had no PTS Cat RC 97 e to a 130 dB SPL tone had a moderate of PTS at all frequencies above 250 H the maximum loss occurring at 20 Cats LC 91 and LC 89 were both expos



Pure tone thresholds and inner ear damage for
xposed to 125 Hz (Fig 1 for explanation)

SPL tone and, after exposure, failed to
d to any signals between 250 and 20 000
o audiometric tests were made at 125

anatomical results for the four animals
ed to 1 000 Hz are shown in Fig 3 Cat
5 had essentially no damage as a result
exposure Cat RC 97 had a double-
d lesion of the outer hair cells and a
narrow region of damage to inner hair
Cats LC 91 and LC 89 both suffered total
iction of OHCs and IHCs throughout all
e apical end of the cochlea

sure to 125 Hz

audiometric results for six animals ex-
l at 125 Hz for four hours are shown in
Cat LC 96 was exposed to a 150 dB SPL
and suffered no PTS Cats LC 71 and LC 77 were exposed to a 155 dB SPL tone and, like LC 96, had normal post exposure audiograms Cat LC 105, exposed to a 157 5 dB SPL tone, had severe PTS for frequencies from 125 to 16 000 Hz, the amount of PTS being greater at the high frequencies Cats LC 88 and LC 70, which were exposed to intensities of 152 5 dB SPL and 155 dB SPL, respectively, failed to respond, after exposure, to tones throughout the range 125 to 16 000 Hz

The anatomical results for the animal exposed to 125 Hz are also shown in Fig 4 Cats LC 77, LC 96, and LC 71 suffered little or no damage from the sound exposure Cat LC 105 had complete loss of hair cells in the basal half of the cochlea and partial loss of cells in the upper turns Cat LC 88 had almost total loss of OHCs and extensive damage to IHCs Cat LC 70 had a nearly normal cochlea, limited dam-

age occurred to outer hair cells at the apical end of the cochlea and to a small percentage of inner hair cells near the apex There was also loss of OHCs in row 3 near the round window

DISCUSSION

Locus of damage—frequency of exposure tone

The relationship between frequency of exposure and locus of inner ear damage is most evident in those animals in which the exposure caused only a narrow region of damage As a result of exposure at 4 kHz, for example, animals LC 76 and LC 110 sustained narrow but severe lesions of both OHCs and IHCs in the upper basal turn of the cochlea In cases in which the exposure frequency was lowered to either 2000 Hz (LC 73) or 1000 Hz (RC 97) however, the region of hair-cell loss occurred at locations further from the basal end of the cochlea (approximately in the middle turn) In animals that suffered severe hair cell destruction after exposure to either 2000 Hz or 1000 Hz (LC 67, LC 89, LC 91), all hair cells were destroyed from the upper middle or lower apical turn to the apical end of the cochlea

Exposure to 125 Hz, in all but one case produced either no damage (LC 77, LC 96, LC 81) or widespread destruction of hair cells (LC 105, LC 88) throughout the cochlea extending from the basal turn to the apical end

Inner ear damage—exposure level

For frequencies of 4000, 2000, and 1000 Hz, the relationship between the amount of inner ear damage and the sound pressure level of the exposure tone is clearly seen the extent of damage was without exception, greater in those animals that received the highest exposure levels

There was greater variability of results for exposure to 125 Hz, nevertheless the two animals with greatest inner ear damage were exposed to sound pressure levels as high or higher than the exposure levels of animals with less inner ear damage

More interesting, perhaps was t amount of change in the exposure required to alter the resultant cochlea from minimal to severe At all expo quencies investigated, for example increase in the intensity of the exposu the difference between moderate existent and severe destruction in the At 125 Hz, a change of less than 5 d exposure intensity sometimes meant ference between a normal and a d cochlea

Hearing loss—exposure level

For exposures to 1000 2000 and 4 hearing loss increased with increase pressure level Again there was great bility in results for animals exposed to

Hearing loss—region and extent of inner ear damage

For animals exposed at 1000 2000 a Hz, the frequency at which maximal loss occurred corresponded well w locus of hair-cell damage Often howe range of frequencies for which hear occurred did not correspond well w width of the cochlear lesion (particula narrow cochlear lesions) Animals wit mal hearing loss at 4000 Hz (LC 76 a 110), for example had damage to both and OHCs in the upper basal turn of the lea Animals with maximal hearing lo kHz (RC 101 and LC 73) had hair ce stroyed in the middle turn of the c (although LC 73 had a second lesion ne apex) The animal with maximal hear at 1 kHz (RC 97) also had lesions in the and apical turns In each of these case range of frequencies at which hearing lo curred was greater than would have bee pected based on the width of the cochle sion or lesions There are several possib planations of this discrepancy The most is that the histological data presented a are based primarily on a determination c

t "presence" or "absence" of each using phase-contrast microscopy No attempt was made to ascertain the n of a hair cell More subtle alterations r cell than its elimination or severe n would not be seen although other es such as electron microscopy might ealed damage sufficient to render the functional This explanation would ount for such discrepancies as rep- by RC 101 which had considerable oss but only minimal hair cell damage ons sustained at the apical end of the of LC 73 and RC 97 may have been d by interruption of the blood supply pical region, a disorder that might be d to the sound exposure (see Bernstein echt, 1967)

cases in which the exposure caused ad destruction of OHCs and IHCs LC 88, LC 91, LC 89, LC 67), there orresponding severe loss of hearing at encies or complete loss at higher fre and severe loss extending into the equency-range In one case, LC 70, exposure hearing loss cannot be ex- n terms of inner-ear damage

loss—destruction
or OHCs?

a obtained in this experiment do not any definitive answer to the question e audiogram reflect the condition of ir cells, outer hair cells or both? Evi rom several animals is relevant to this n Cat LC 88 had almost complete de- n of OHCs but a large number of IHCs apical turn were still present, post- re testing indicated complete loss of LC 91 also had complete loss of hear ough some IHCs remained in the apical sal turns RC 101 had post exposure loss for frequencies from 2000 to Hz although IHCs remained throughout hlea

above cases (LC 88, LC 91, and RC 101) might be taken as evidence that the audiogram does not necessarily reflect the condition of the IHCs

In contrast to the above cases, LC 73 had normal hearing for low frequencies despite the nearly total destruction of OHCs in the apical turn, IHCs were intact in the apical turn and throughout the cochlea except for a narrow region in the middle turn In this case, it might be argued that the audiogram does reflect the condition of the IHCs

Finally, in considering the results of the present study and of many others that have related hearing loss to inner-ear damage, the limitations of methods used in assessing *inner-ear damage must be kept in mind* No single histological technique provides a complete assessment of intracochlear damage or change resulting from an exposure It is neces sary to accurately assess damage to hair cells, intracochlear changes such as mechanical de *struction of nerve fibers, disruption of the* synaptic region between sensory cell and nerve fiber, interruption of blood supply, and damage to supporting structures

There are also limitations in the methods used to measure changes in hearing in experi mental animals Occasional anomolous results may be expected In an animal with a severe hearing loss, it may be difficult to obtain threshold measurements For a normal animal or one with moderate hearing loss, tests of absolute threshold may be started by presenting a tonal signal well above threshold and decreasing it until the animal no longer makes a behavioral response For animals with severe hearing loss, it may not be possible to use a tonal stimulus that is much above threshold Therefore great care must be taken not to produce 'neurotic' behavior by creating highly stressful conditions for an ani mal—particularly one that has been exposed to loud sound or has otherwise been treated so as to produce a severe hearing deficit The presence of tinnitus may also disrupt the ani mals performance in response to weak tonal signals

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ZUSAMMENFASSUNG

Katzen wurden reinen Tönen von 125, 1000, 2000 und 4000 Hz mit einer Schallintensität im Bereich von 120 bis 157,5 dB SPL, während einem Zeitraum von 1 Std (1000, 2000 4000 Hz) bzw. 4 Std (125 Hz) ausgesetzt Vor und nach jedem Versuch wurde von jedem Tier ein Tonaudiogramm erstellt Nach Schallexposition wurden die Hörtests so lange wiederholt, bis feststand, dass die normale Horfähigkeit wieder erlangt war oder ein bleiben der Hörverlust festgestellt werden konnte Die Cochlea der Tiere wurde im Phasenkontrastmikroskop untersucht Der Zustand aller Haarzellen wurde untersucht und ausgewertet Das Ausmass des Innenohrschadens und die Frequenzbreite, in der ein Hörverlust auftrat, wurden grösser, wenn die Tiere Tönen niedriger Frequenz ausgesetzt wurden Beispielsweise wurde beobachtet, dass eine Schallexposition von 4000 Hz Haarzellschaden in einem kleinen Abschnitt der Cochlea hervorrief und einen Hörverlust in einem eng begrenzten Frequenzbereich verursachte Nach Exposition von 125 Hz wurde ein ausgedehnter Innenohrschaden gefunden mit Hör[illegible] Frequenzbereich von 125–6000 Hz

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W D Neff Ph D
Psychology 320
Indiana University
Bloomington Ind 47401 USA

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AUDIOLOGICAL FINDINGS IN 125 CASES OF ACOUSTIC NEUROMAS

J Thomsen and K Terkildsen

From the University ENT and Neurosurgical Departments Rigshospitalet Copenhagen Denmark

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The audiological findings in 125 patients with confirmed acoustic neuromas are presented the classification by Pulec et al (1971) we ned um size and 105 large tumours small tu e not represented A clear connection between of hearing loss and tumour size was noted e was no correlation between duration of l tumour size An attempted evaluation of the udiological tests (ABLB and Metz recruitment h discrimination scores tone decay reflex de ekesy tracings) that were applied to the pa been made Attention is called to some audio-lings which to our knowledge have not been previously No patient in the entire material l hearing 73 had anacusis and 52 hearing arying degree In the presence of a normal al ear the evaluation of audiological tests at holds poorer than 80 dB is rather questionable was therefore concentrated on 32 patients ring loss of 80 dB or less The pathophysio-s for the typical hearing loss in patients with ar disease is a reduction in the number of s in the acoustic nerve and it was to be ex abnormal findings would be present especially ests that exert the greatest demand on the mission capacity of the nerve in other words pplication of intense and/or prolonged sounds test is just such a procedure and it is not sur t it shows the highest degree of validity be he tests No single test suffices to distinguish rom retrocochlear disease and it is necessary to ery of tests Any unexpected variability in the of ordinary routine test results has gradually ne main indication to pursue the diagnosis with orate procedures and it has been a great help apply the Metz test as a part of our routine amination

s a close connection between the size oustic neuroma and the complications, g mortality at operation Early diagnosis is vital for the patient Though much improved over the past 25 years, our efforts to detect these tumours at an early stage have by no means paralleled the advances made in surgical treatment of acoustic neuromas This is very regrettable since in most cases it is now possible to remove small tumours with very low mortality and little or no damage to the facial nerve

This paper presents the audiological findings in 125 patients with surgically confirmed acoustic neuromas We have attempted an evaluation of the usefulness of the various audiological tests that were applied to the patients Attention is called to some of the audiological findings, which to our knowledge have not been described previously We also present a case history, with an—unfortunately—not infrequent course

stigation was supported by a grant from the P rsen Foundation (Grant B 921)

METHODS

The classical ABLB-test continues to be a very important tool in the differential diagnosis of perceptive disorders and it was applied systematically to all patients where the hearing loss did not exceed 80 dB PTA

The Metz recruitment test, based on the middle-ear muscle reflex thresholds, was introduced in the world literature from this department in 1952 (Metz, 1952) Over the years it has been applied with several types of

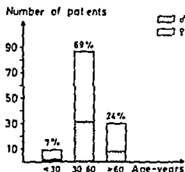


Fig 1 Age distribution of 125 patients with acoustic neuromas

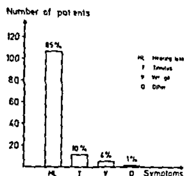


Fig 3 First subjective symptom in 1[illegible] acoustic neuromas

equipment to all patients unless middle-ear pathology rendered it unsuitable

Speech audiometry was not readily available in our department before 1962 and was omitted in some patients early in the series Other tests were used more sporadically, mostly as a part of specific time limited scientific projects, such as the Békésy audiometry procedures and the SISI-test, as described by Jerger (Jerger et al, 1959), various types of tone-decay testing (Carhart, 1957, Owens, 1964, Sørensen, 1962), and now in recent years the reflex-decay test (Anderson et al, 1969)

MATERIAL

The material consists of 125 consecutive patients with acoustic neuromas, operated on in the Department of Neurosurgery, University Hospital (Rigshospitalet), in the period 1957–72 Exact measurements of the tumour size were difficult to obtain, and there are often discrepancies in the way o[illegible] neurosurgeons interpret the findi[illegible]

In the following, we have adopt[illegible] fication described by Pulec et al [illegible] tumours (intracanalicular) are co[illegible] internal auditory canal *Medium s*[illegible] extend into the cerebello-pontine [illegible] without fifth nerve involvemen[illegible] should be no symptoms from the [illegible] the cerebellum In *large* tumours [illegible] nerves are involved, causing [illegible] brain stem symptoms The intra[illegible] sure may eventually be increase[illegible] to these criteria there were 20 m[illegible] and 105 large tumours in our [illegible] small tumours were not represented

The age distribution is seen in F[illegible]

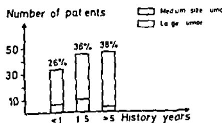


Fig 2 Length of history in relation to tumour size in 125 patients with acoustic neuromas

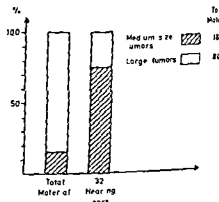
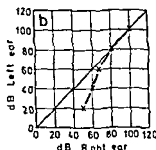
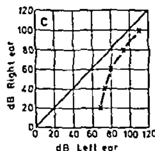


Fig 4 Distribution of tumour size in total ma[illegible] patients with acoustic tumours compared [illegible] size in the selected material of 3? patients [illegible] ears

x
x
20 40 60 80 100 120
dB Right ear





ABLB recruitment test results in 3 patients with
Metz recruitment test

ty of patients fell within the 30–60 year
oup, tumours were rare below 30 years
Women outnumbered men by two to

ig 2 the duration of symptoms is related
nour size Only 26% of patients were
ned within the first year and 38% had a
y exceeding 5 years There is a tendency
shorter history for the medium sized
rs, but the variability is large and there
certain connection between length of
y and tumour size

3 categorizes the first symptoms re
by the patients according to type In
hearing loss was the initial symptom,
entioned tinnitus and 4% vertigo The
ning one patient reported an initial re-
facial sensitivity There were 7 bilateral
rs, and while neuromas as a whole are
low the age of 30 (Fig 1) in our series
% we found that 4 out of 7 patients with
ral neuromas were below that age All
4 houng patients had Recklinghausen's
se while the remaining 3 had no signs of
lisease A total of 6 patients suffered from
urrence of the neuroma (5%)

patient in the entire material had normal
hearing Of the 125 patients, 73 had anacusis, and 52 hearing loss of varying degree This paper is mainly concerned with the various audiological tests applied and we decided for practical reasons to concentrate our attention on 32 patients with a hearing loss of 80 dB or less

In the presence of a normal contralateral ear, we consider the evaluation of audiological tests at PTA thresholds poorer than 80 dB to be rather questionable

While there is a great preponderance (84%) of large tumours in the material as a whole, the medium-sized tumours dominate (75%) in the selected material (Fig 4) Thus there is a clear connection between the degree of hearing loss and tumour size This is in contrast to the lack of correlation between duration of history and tumour size

The 32 patients had a mean PTA air conduction threshold of 54 dB There was good concordance between the SRT (Speech Reception threshold) and the PTA

e I *Speech discrimination scores in 24 nts with acoustic tumours*

	0–20%	20–40%	40–60%	60–80%	80–100%
er	10	2	3	5	3
	60 dB	42 dB	58 dB	50 dB	45 dB

954

Table II *ABLB and Metz recruitment tests in 30 patients with surgically confirmed acoustic tumours*

ABLB		Metz	
+recruitment	−recruitment	+recruitment	−recruitment
	23		23
4		4	
3			3
23%	77%	13%	87%

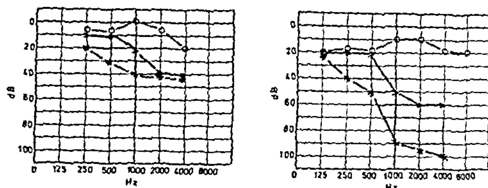


Fig 6 Pure tone audiograms obtained with an interval of one month illustrating the threshold shift when masking the non tumour ear

Table I shows speech descrimination scores (SDS) in 24 patients There is no relation between SDS and PTA Only 8 patients (33%) had speech descrimination scores better than 60%

In Table II the ABLB and Metz recruitment tests are compared in 30 patients, 7 (23%) of the patients had positive recruitment with the ABLB test Of these 7 patients 4 also exhibited recruitment by the Metz test In the remaining 3 patients there was a distinct discrepancy between the findings by the two methods, in favour of the Metz method The results of the ABLB tests in these 3 patients are illustrated in Fig 5*a* *b* *c*

Patient a A 53 year old woman with a progressive hearing loss in the right ear for 4 years She had been dizzy for about 1 year prior to the first examination The ABLB test shows positive recruitment of the delayed type At surgery she had a large acoustic neuroma with impression in the brain stem

Patient b A 16-year old man with history of hearing loss and tinnitus in ear There was no vertigo The Al showed complete recruitment A larg was removed from the cerebello-pon le, it had caused some impression on stem

Patient c A 26-year-old woman history of hearing loss for 1½ years a interference with the facial sensitivity side She showed a classical rec phenomenon at ABLB There was a size tumour but no impression on th stem

Adaptation tests

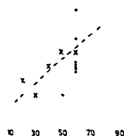
Fifteen patients were examined for th ence of tone decay Eleven of these (73 a type of decay typical for retrocochl ease while the outcome was negati (27%) The results from Bekesy tracing patients are seen in Table III 30%

Table III *Classification of Bekesy tracings in 10 patients with acoustic tumours*

Type	No	%
I	1	10
II	2	20
III	3	30
IV	4	40

Table IV *Patients with positive res audiometric tests consistent with site of*

Test	No tested	Per cent (%)
Speech discrimination	24	58
ABLB	30	77
Metz	30	87
Tone decay	15	73
Reflex decay	5	60
Bekesy audiometry	10	70



PTA threshold shifts in relation to hearing loss in ear with no masking of non tumour ear

or II tracings, while the remaining 70% of type III and IV

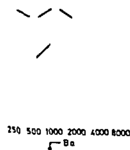
e patients could be tested for stapedius decay (Anderson et al , 1969), 3 had a decay, but in 2 patients the reflex at 500 mained stable for a period of 10 sec

IV shows the results of the six audio- tests that were applied in this series ercentages of correct results, being con t with presence of a retrocochlear type ion are indicated It is obvious that the recruitment test shows the greatest ty in the diagnosis of the retrocochlear ng loss

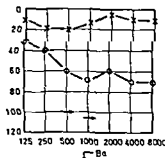
15 of the 32 patients we observed a derable threshold shift during pure tone metry when masking was applied to the alateral ear This is illustrated in Fig 6 where two pure tone audiograms obtained in one patient with an interval of one month are shown This case serves to illustrate the great threshold shift that may occur with adequate masking of the contralateral ear (overhearing to the unmasked ear is out of the question), and also the big difference in audiograms even within such a short interval This is a characteristic feature which we observed quite frequently in pure tone audiometric examinations of patients with acoustic neuromas

Fig 7 shows the number of PTA threshold shifts that occur with adequate masking of the non tumour ear, as related to the unmasked PTA thresholds in 15 patients There is a correlation between shift of threshold and the level of the unmasked PTA, the poorer the unmasked PTA threshold, the greater the threshold shift with contralateral masking

In 2 patients we noticed a symptom which to our knowledge has not been reported previously (Fig 8) The contralateral left ear was normal in both patients, but nevertheless it was impossible even with the use of maximum tonal stimuli at this ear to demonstrate the presence of a middle-ear reflex on the tumour side On stimulation of the tumour ear a muscle reflex was found in the normal ear, but as might have been expected, only with stimulus intensities above normal

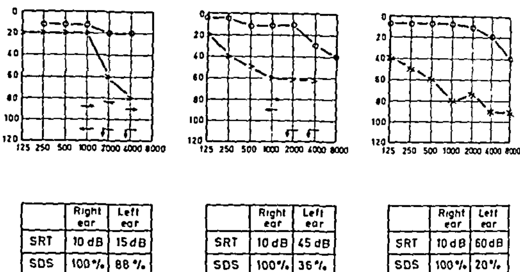


	Left ear	Right ear
SRT	10 dB	40 dB
SDS	92 %	8 %



	Left ear	Right ear
SRT	15 dB	60 dB
SDS	95 %	36 %

Fig 8 Audiograms from 2 patients with acoustic neuromas and normal non tumour ear No middle ear reflex could be demonstrated in tumour ear even with maximum tonal stimuli in the non tumour ear



	Right ear	Left ear
SRT	10 dB	15 dB
SDS	100 %	88 %

	Right ear	Left ear
SRT	10 dB	45 dB
SDS	100 %	36 %

	Right ear	Left ear
SRT	10 dB	60 dB
SDS	100 %	20 %

Fig 9 Audiograms in patient with acoustic neuroma (case history)

In one patient this absence of muscle reflex on the tumour side, together with the need to use abnormally high stimulus intensities on this very side before a reflex could be recorded in the normal ear, initially made us suspect that she suffered from unilateral otosclerosis accompanied by a considerable perceptive component

Finally we want to report a case history with a course that unfortunately may occur not infrequently The patient was a 54-year old woman When we first saw her, she had noticed a slight hearing loss and tinnitus in the left ear for 4 months The audiogram is seen in Fig 9 The differential-caloric test, taste and naso lacrimal-reflex examination, corneal and facial sensitivity, were all normal There was pronounced recruitment at ABLB test and no tone-decay Discrimination scores were high The only abnormal finding was absence of recruitment as measured by the Metz test The case was regarded as one of Meniere s disease and it was decided to re examine the patient after one month Because of lack of symp toms, however, she failed to report until after the lapse of 6 months She had then experi enced a rather sudden onset of severe vertigo The audiogram, shown in Fig 9 demonstrated a deterioration of hearing Caloric reaction had disappeared and the naso lacrimal reflex was also impaired Taste examinations were still normal There was recruitment at ABLB and the Metz test now represents a borderline case Other findings were pronounced tone-decay and a Békesy type IV tracing Iophen-dylate cisternography was thought to dem-onstrate an intracanalicular tumour This was removed by the suboccipital approach and was found to be of medium size extending 1 cm into the cerebello pontine angle During the operation the facial nerve became injured, resulting in total paralysis, but otherwise the course was uneventful

DISCUSSION

Nowadays the reliability with which so-called recruitment tests are able to differentiate be-tween cochlear and retrocochlear lesions is being re evaluated We like to think that this doubt as to the diagnostic validity arises be-cause we now see the patients at an earlier stage of the disease This seems not to be true in the present series however, where the tumours were as large as ever, when seen at surgery Still we find the same ambiguity in the

sults as reported by others A common s the considerable test-retest variability individual patient

crux of the matter appears to be the fact is usually quite apparent, when an ear is ting but far more difficult to prove that tment is not present Patients with retro-ear lesions become grouped with those lo not cooperate to a satisfactory degree uite often it is the examiner's first im on that he is faced with malingering considering the individual tests it is ap-iate to distinguish between those that are icted at the threshold of hearing, and threshold procedures

e tone audiometry will always be the first ostic step Even at this stage the true osis may be suspect because of con ible uncertainty about the result The nt will often report that it requires some al effort to concentrate on the detection eshold intensities and the introduction of moderate masking to the contralateral ear s a more than normal degree of so-called al masking effect This is in contrast to ear lesions where threshold audiograms eproduced with great accuracy These igs have led us to the routine of recording gr ims both without and with adequate ing

kesy audiometry is another threshold dure In 30% of the patients who were itted to this test the tracings were of the s that are conventionally associated with a lear type of disease thus agreeing with indings of Johnson (1968) Here again the duction of masking may change the out of the test (Blegvad 1968) Usually nts with acoustic neuromas have a pure iteral sensorineural hearing loss and of a magnitude that it is appropriate to mask contralateral normal ear It is our impres that just this masking is a main cause of variability in the outcome of threshold test edures that is so characteristic of these ents The most widely used suprathreshold edure is the classical ABLB test which was applied whenever possible Here again the outcome will often be equivocal and the patient experiences great difficulty in deciding on levels that are equally loud binaurally We agree with Simmons & Dixon (1966), that this may lead an "examiner who is naive or in a hurry to find an intensity—any intensity—which the subject will call equal to the reference ear" In some of our patients the uncertainty in this respect was so pronounced that it was irrelevant to give specific figures for the ABLB test A positive outcome of the ABLB test indicating a cochlear lesion was found in 23%, which again is in close agreement with Johnson (1972)

Speech discrimination scores constitute another important suprathreshold measurement This test suffers from lack of standardization and any evaluation rests on rather subjective norms for the amount of discrimination that might be expected at various levels of sensori neural loss Even on this basis we found the test to be very valuable especially in patients where the PTA was only moderately reduced Here too the application of contralateral masking would often cause the performance to deteriorate considerably The pathophysiological basis for the typical hearing loss in patients with retrocochlear disease is a reduction of the number of active fibres in the acoustic nerve and it was to be expected that abnormal findings would be present particularly in those tests that exert the greatest demand on the total transmission capacity of the nerve in other words with the application of intense and/or prolonged sounds The Metz test is just such a procedure and it is not surprising that it shows the highest degree of validity between all the tests There are really no firm criteria for the evaluation of any of the tests mentioned here, this applies also to the Metz test We used to say that a gap of more than 60 dB between the threshold for hearing and the reflex threshold signifies lack of recruitment If this is accepted without reservation any very slight cochlear loss should be categorized as being retrocochlear,

for in essence the reflex threshold as measured in HL remains unaffected in patients with cochlear lesions It is only when we reach magnitudes of hearing loss around 60 dB that the reflex threshold begins to increase This is in contrast to patients with acoustic neuromas where even a very slight hearing loss will be accompanied by a very significant shift of the reflex threshold This is particularly true for the medium to high frequencies Sometimes there may be a reflex at 500 Hz at intensities that are not quite 60 dB above the subjective threshold This is exemplified in our case history where we first found no recruitment and later a borderline case of recruitment

As experience accumulates with the Metz test, it should be possible to establish a better criterion than the 60 dB gap and this will most certainly have to involve a distinction between findings at the various frequencies

So far the general experience with the reflex decay test is quite sparse, but it should be noted that out of 5 patients with surgically confirmed neuromas, where this test was applied, 2 did not show any decay

The absence of middle ear muscle reflexes on the tumour side in spite of a completely normal stimulus ear contralaterally has not been reported previously There is no obvious explanation for this phenomenon, which was present in 2 patients It seems far-fetched to suggest that it signifies an early stage of facial nerve involvement Because of the isolated character of this symptom, a nuclear brain stem type of impairment appears more likely

In our final conclusion, we can only agree with others that no single test suffices to distinguish cochlear from retrocochlear disease and that it is necessary to use a battery of tests The question remains when to apply this time-consuming battery It is important to maintain a constant awareness of the possible presence of retrocochlear disease To us, any unexpected variability in the outcome of ordinary routine test results has gradually become one main indication to pursue the diagnosis with more elaborate procedures It has also been a great help always to apply the Metz [illegible] as a part of our routine clinical examination

ZUSAMMENFASSUNG

Die audiologischen Befunde von 125 Patienten [illegible] operativ bestätigtem Acusticusneurinom werden [illegible] [illegible] wicklungszeit und Grösse des Tumors Es wurde [illegible] sucht den Wert folgender audiologischer Teste zu [illegible] teilen Fowler's und Metz Recruitment Test Sp[illegible] audiometrie Tonedecay Test Reflexdecay Test [illegible] Békésy Audiometrie Es wird auf einige audiolog[illegible] Wahrnehmungen aufmerksam gemacht die unseres [illegible] sens bisher nicht beschrieben worden sind Im gesa[illegible] Krankengut kam keine normale Gehörfähigkeit vo[illegible] [illegible] problematisch wenn die Toenaudiogrammst[illegible] dB oder schlechter beträgt Deshalb haben wir [illegible] Aufmerksamkeit auf die Patienten konzentriert [illegible] Hörverlust 80 dB oder kleiner ist Die p[illegible] physiologische Ursache des typischen Hörverlust[illegible] Patienten mit retrocochlearem Leiden ist eine Redu[illegible] der Zahl aktiver Fasern im Nervus acusticus Es [illegible] [illegible] totale T[illegible] beanspru[illegible] und/oder [illegible] dauernden Lauten getestet wird Gerade Metz Te[illegible] dies und es kann nicht überraschen dass er unter [illegible] Tests der zuverlässigste ist Ein einzelner Test g[illegible] nicht um zwischen cochlearem und retrocochle[illegible] Leiden zu unterscheiden Es ist daher erforde[illegible] mehrere Teste zu verwenden Jeder unerwartete Be[illegible] bei normalen Routinetests ist allmählich eine H[illegible] [illegible] Hilfe immer den Metz Test zu verwenden

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J Thomsen M D
University ENT Department
Rigshospitalet
Blegdamsvej 9
DK-2100 Copenhagen
Denmark

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A MODIFIED "SOFT SURFACE SPECIMEN TECHNIQUE" FOR EXAMINATION OF THE INNER EAR

A. Axelsson, J Miller and B Larsson

From the Department of Otolaryngology and the Regional Primate Research Center University of Washington Seattle, Wash USA

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Abstract A new surface preparation method was devised for assessing intracochlear vessels and sensorineurepi thelium The method is a combination of the conventional osmic acid stained surface preparation method and a surface preparation technique with injection of Prussian blue and decalcification of the cochlea Such a combina tion preserves most advantages of each and eliminates many of the disadvantages To preserve the delicate sensory and nervous structures we examined variations of the preparation, paying particular attention to fixation staining and decalcification procedures and solutions Varying the preparation had a less marked effect on exter nal wall structures than on the basilar membrane the primary changes consisted of variation in staining con trast The method proved useful for demonstrating the normal anatomy and pathological changes in cochlear vasculature and in sensorineural and supporting structures of the labyrinth in light and phase contrast microscopy at moderate magnifications

We have previously reported preliminary histological observations made with a modified surface preparation technique based upon the combination of two techniques the osmic acid staining of undecalcified temporal bones and the Prussian blue injection and subsequent decalcification of the cochlea (Axelsson et al , 1974) We have now studied a large number of cochleas from guinea pigs, chinchillas, cats and monkeys with this technique, using both normal and experimental pathological material We report here in more detail the prepara tive steps and compare the present met with related ones

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METHODS

The study of procedural variations emplo 50 guinea pigs with a readily evoked Prey reflex In addition, 100 guinea pigs, 6 rhe monkeys, 13 cats and 11 chinchillas were u for studies of normal cochlear vasculat of the

changes, influence of noise and/or sa and influence of experimental acou tumors

Some preparation steps were stable others varied (Table I and Fig 1) The ba techniques are reported elsewhere osmic a undecalcified surface preparation (Engstr et al , 1966, Johnsson & Hawkins, 1967) Pr sian blue decalcified surface preparation (elsson, 1968, 1972) and a modification e ploying both these techniques (Axelsson et 1974) Basically, the sequence of preparati steps included (1) vascular contrast injectio (2) fixation, (3) osmic acid staining (4) calcification, and (5) slide mounting of d sected material

Perfusion and contrast injection

Perfusion of saline and injection with Pruss blue are described in detail previously (Axel

Variations (preferred technique italicized)

	Mode	Time
fixation		
Millonig)	*Intracochlear inj*	*0.5* to 15 min
id (0.5%) pH≈6.5	Immersion	
aldehyde pH≈5 0	Intracochlear inj	2 to >24 hours
s solution pH≈7 4	and immersion	*24 hours*
aldehyde pH≈4 0	*Immersion*	
ATION		
Nitric acid pH<1	*Immersion*	*as required*
A H_2O buffered NaOH pH≈7 4		
DTA H_2O not buffered, pH≈5 0		
A Millonig pH≈6 0		
pH<1		
AND DECALCIFICATION		
A in 2% glutaraldehyde pH≈5 0	Intracochlear inj and immersion	as required
A in Karnowsky s solution pH≈6 0		
STAINING**		
(Millonig) osmic	Intracochlear inj	3 5 min
5%) pH≈6.5	*Immersion*	5 *10* 15 min

* Omega Chemical Corporation Cold Spring on Hudson N Y
osmic stained previously

3, 1972) Static pressure was constant as was the injection solution with to strength and temperature Amount of ' solution varied with size of the ani- the guinea pig and chinchilla the entire system of the animal was perfused iected In the cat and monkey the de aorta was clamped

ing vascular perfusion and removal of poral bones, the oval and round ws and the apex of the cochlea were illy opened in some of the guinea pigs chlear structures were fixed either by injection and subsequent immersion in ve or by immersion only Cochleas fixed with either Karnowsky's solution, acid, or glutaraldehyde, or a combina- the latter two Using osmic acid, the leas were stained simultaneously Some eas fixed with Karnowsky's solution and glutaraldehyde, were decalcified, divided longitudinally and subsequently counter- stained with osmic acid

Decalcification

After fixation the temporal bones were de- calcified Time varied with content and strength of solution from a few hours to five days The decalcification agent was changed *daily to a fresh solution In some guinea pigs* the temporal bones were examined after simultaneous fixation and decalcification

In all ears, following decalcification the cochlea was washed in tap water for 2 hours, dehydrated in successive stages of increas- ingly concentrated alcohol, and stored in glycerin Details of the order and variations in the procedures are given in Table I and Fig 1

Dissection

The dissection began with a midmodiolar or paramodiolar section made close to the round

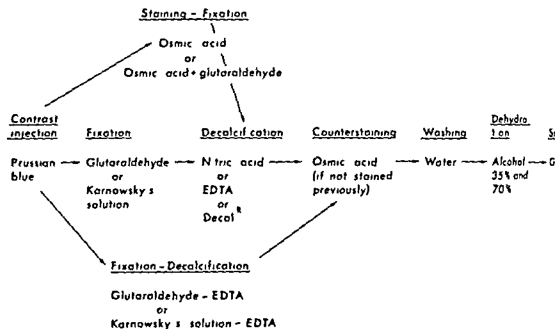


Fig 1 Procedural variations of the present soft surface specimen technique

window without affecting it With the sectioned surface facing the microscope, the specimen was examined for such changes as distension or collapse of the vestibular membrane, occurrence of hemorrhage or debris, etc Transverse cuts were then made with a razor blade on the apical and basal side of the spiral lamina in each turn Spiral lamina pieces together with the attached modiolus parts were then removed by simply pulling them away from the external wall with a watchmaker's forceps The basilar membrane of the basal turn adheres more firmly than in the other turns and must often be divided from the external wall by careful sections The vestibular and tectorial membranes are removed from the specimen with a watchmaker's forceps stabilizing the specimen with manual pressure of a cover slip glass under stereomicroscope magnification of ×50

The remaining parts of the modiolus structures were removed by apico basal sections Such pieces contain VIII nerve fibers, spiral ganglion cells, the modiolus wall, radiating arterioles, collecting venules and the artery and vein in the modiolus

The remaining membranous external on the inside of the cochlear bone w moved with a delicate spoon shaped ment by blunt dissection between the b the spiral ligament

All dissected pieces were placed on slides in glycerin and cover slipped

Microscopy

In general, vasculature was studied unde microscopy, sensorineuroepithelium tectorial and vestibular membranes a vessel wall under phase contrast micros The spiral lamina was examined from the side, visualizing the peripheral va arcades and nerve endings, and from the side visualizing the sensory and supp structures and the limbus vessels The specimens are sufficiently elastic and fir they can be turned without damage

The external wall appeared to be little enced by the variations in the prepar steps We focused therefore, on presen and visualization of the sensory structur the organ of Corti



uinea pig second turn organ of Corti longitudi on l ght microscopy After the mid-modiolar sec made in the decalcif ed cochlea the sectioned surface is examined before further dissection OHC=outer hair cells TM=tectorial membrane TL= tympanic lip SL=spiral limbus

RESULTS

Technical Aspects

st injection

viously the Prussian blue injections did ve consistently uniform vessel injec The basal turn was injected most easily e apical turn least well In certain prep ns some vascular tributaries were less njected than others for no apparent 1

on and staining

btained adequate fixation with all the tions adopted We preferred the gluta hyde solution, however because it easier to prepare The osmic acid pre d two disadvantages in both staining and on (1) For an even osmic stain through he cochlea the osmic acid has to be in d through the opened cochlea Contrarily, the opened cochlea showed little evidence of insufficient fixation after immersion in glutaraldehyde (2) The time necessary for adequate fixation with osmic acid is longer than that for adequate staining of the tissue Consequently, osmic acid must be removed from the cochlea or else the tissues stain too darkly obscuring the vasculature Glutaraldehyde injected following osmic acid fixation staining both stopped further staining and also ensured adequate fixation

A particularly fast variation of this proce dure combined fixative and decalcification agent in one preparative step However, the sensory epithelium was not as distinct with this approach Other variations may be used to advantage for different purpose For studies of vascular anatomy Prussian blue injected ma terial may be either unstained or lightly in jected with osmic stain For investigations of discrete pathological changes windows and

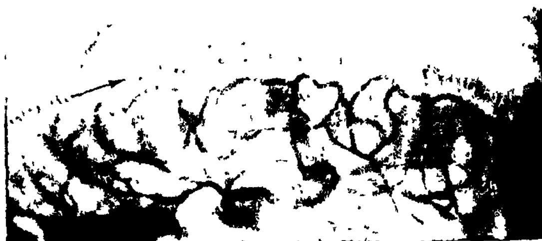


Fig. 3. Guinea pig, basal turn, spiral limbus, transverse section light microscopy. The limbus vessels are most easily demonstrated with an apical view. Arrow=Huschke's dents.

apex should be opened, but injection of fixative or staining solutions should be avoided. In such cases the cochlea may be immersed in glutaraldehyde and examined after decalcification-sectioning but before counterstaining for gross changes and after osmic-counterstaining for discrete changes.

Decalcification

Tissues decalcified in EDTA showed little significant difference from those decalcified in a weak solution (0.5%) of nitric acid. Gross distortion of the structures of the basilar membrane, evidenced by a wavy appearance of the organ of Corti, often occurred with Decal®, and sometimes with EDTA. The distortions could not be correlated with any obvious preparation variable (i.e., inadequacy of fixation, fixative pH, etc.). Definitely, best results were obtained when the decalcification solutions were changed daily.

Counterstaining

The primary precaution to be taken with osmic acid staining is control of exposure time. The tissue appears to be considerably more susceptible to overstaining when it is fresh than following fixation and decalcification. It is difficult to escape the concern that mec[...] cal damage to cochlear structures ma[...] caused by direct injection of fixati[...] staining solutions.

Varying the preparation steps had a marked effect on external wall structures on the basilar membrane, particularly th[...] gan of Corti. The primary changes consist[...] variation in staining contrast. Many o[...] initially observed differences thought t[...] due to different preparation steps were f[...] to be minimal as experience was gained this approach.

Photography

Using optical sections with phase co[...] microscopy, the spiral lamina can be exam[...] in a midmodiolar view (Fig. 2) and from apical and basal aspects (Figs. 3–5). The a[...] aspect provides the clearest view of the li[...] vessels (Fig. 3) and limbus cells and pe[...] study of the stereocilia of inner and outer cells, the reticular membrane, pillar cells [...] 4), myelinated nerve fibers (Figs. 5–6) a[...] supporting cells. The basal aspect pro[...] clear views of the vessel of the ba[...] membrane (vas spirale, outer spiral ve[...]

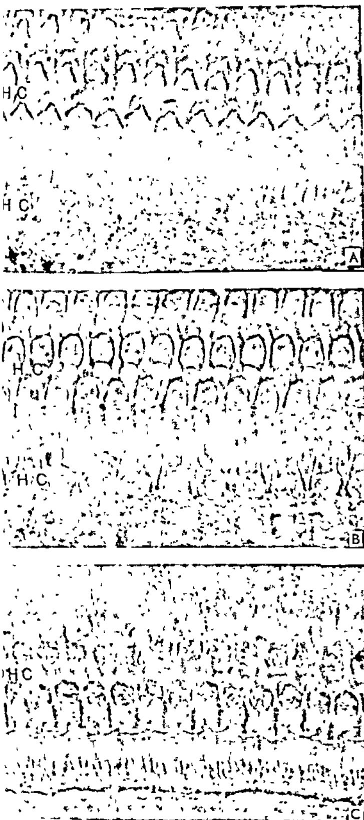


Fig 4 Guinea pig, basal turn, basilar membrane, transverse section, apical view, phase contrast microscopy With the aid of optical sections, different structures in the organ of Corti can be visualized. (*A*) Stereocilia of the outer hair cells (*OHC*) (*B*) The reticular lamina as well as the inner hair cells (*IHC*) (*C*) The cell bodies of the outer hair cells, the stereocilia of the inner hair cells, and pillar cells

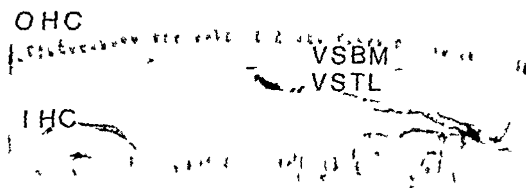


Fig 5 Gu nea p g basal turn sp ral lam na transverse section light microscopy The vessels in the sp ral lam na are more clearly seen with a basal view than with an ap cal view The myelinated nerve end ngs are clearly seen in both v ews *OHC*=outer hair cells *IHC*= in *VSBM*=vessel of the bas lar membrane *VST* the tympanic lip

F g 6 Gu nea p g basal turn bas lar membrane trans verse section basal v ew phase contrast m croscopy Adopting high magnif cation and phase contrast the ves sel lumen and wall are eas ly v sualized n relat on to other anatom cal structures *OHC* outer hair cel inner hair cells *VSBM*=vessel of the basilar n *VSTL*=vessel of the tympanic l p



umea pig basal turn external wall longitudinal ght microscopy The capillaries of the stria vas *VS*) are visualized as well as the vessel(s) of the ominence (*VSSP*) and the collecting venules

vessel of the tympanic lip (Figs 5–6) ords a different and very clear view of elinated nerve fibers, the radial fibers nel bundles, and the nuclei in the inner ter hair cells A fairly common finding dividual differences in 'quality' of the y and supporting cells In a given prep- hair cells could be quite clear, while hout that preparation pillar cells were stinct, in another preparation the oppo- ight be found etc

present method permitted visualization tudy of the external wall vasculature arable to that afforded by Prussian blue ut osmic staining (Fig 7) Using different l sections we viewed the vessels facing the endolymph and perilymph and selectively studied the vessels in the deeper part of the spiral ligament In both injected and unin jected preparations of the external wall we observed the vessel lumen, its wall, perivascu lar spaces, pericytes, etc (Fig 8), and studied the surface cells of the stria vascularis (Fig 9) We also easily observed the structure of the vestibular and tectorial membranes This method proved equally useful for study of the vestibular membranous labyrinth including both vasculature and neural structures (Figs 10–12)

We have used the present method to study discrete pathological changes in membranes and lymphs, in hair cells, nerve endings, vas- culature and supporting structures that re sulted from mechanical and electrolytic le- sions of the cochlea (Hallen et al , 1974 *a*, *b*), from noise exposure (Lipscomb et al , 1974), and from middle ear pressure changes (Lam kin et al , 1974)

DISCUSSION

Five principal methods have traditionally been used to visualize intracochlear structures

Serial sections after conventional staining, examining the longitudinal section (e g Guild, 1919 1932, 1937, Schuknecht 1953, 1959, Ward & Lindsay, 1964)

Surface techniques with staining of the in- traluminal blood (e g Scuderi & del Bo, 1952, *Smith, 1954, Maass, 1969*)

Surface techniques with staining of the vascu lar wall (e g Smith, 1951, Nomura & Hiraide, 1968, Kirikae et al , 1969, Sugar et al , 1972)

Surface techniques with osmic staining (e g Retzius, 1882, Neubert, 1950, Engstrom et al , 1963, 1966, Spoendlin, 1966, Johnsson & Hawkins, 1967, Kirchner, 1968, Bohne, 1972)

Surface techniques with injection of different contrast media or intracochlear precipitation of stain (e g Eichler, 1892, Siebenmann, 1894, Naheya 1923, Smith 1951, 1953, 1954,



Charachon, 1961, Axelsson 1968 ' 1970, Hansen & Mazzoni 1970)

Most of these methods have been relation to possible decalcification embedding of the material and type calcification and embedding agent been assessed by light and phase and lately by electron and scanning scopy Sometimes all five of these have been adopted on the same

The present method is a surface method introduced by Retzius forgotten and revived by several recent decades (Neubert 1950 E al 1966 Johnsson & Hawkins I cochlear vasculature is visualized by of a contrast material Prussian sensorineurepithelium is stained by acid and the temporal bones are Consequently, bination of the currently much used (undecalcified) osmic staining technique the 'soft surface (decalcified) Prussian



Fig 8 Guinea pig basal turn scala vestibul longitudinal section phase contrast microscopy In the contrast injected preparation (*A*) the occurrence of avascular channels (*AVC*) perivascular spaces (*PVS*) and pericytes (*PC*) as well as the vessel wall can be studied In the un prepanation (*B*) under these high magnification these features may be studied in addition to the adequate visualization of the vessel lumen

9

10

9 Gu nea p g basal turn stria vascularis longitudi sect on phase contrast m croscopy In preparations h unsuccessful contrast inject on the vessels and their ent are visual zed in relation to the surface cells

Fig 10 Gu nea p g macula sacculi vessels injected by contrast are visualized in relation to the nerve f bers

75*954



Fig 11 Guinea pig posterior ampulla The vasculature of the ampulla wall and the canal are visualized in relation to the crista (*arrow*)

surface technique Table II is a list of the principal advantages and disadvantages that we see in each of the surface preparation procedures

We suggest, at least in some reg almost cavalier attitude in this appro tissue is quite resistant to damage an handled, turned and coverslipped fre

Table II *Comparison of the surface preparation techniques*

Prussian blue decalcification	Hard surface preparation technique (osmic only)	Soft surface preparation technique (Prussian blue plus osmic)
Advantages	*Advantages*	*Advantages*
Fast	Vessel wall and lumen assessed	Fast
Easy dissection	Sensory and nervous structures seen at high magnification	Easy dissection
High contrast	Good for pathology	Whole cochlea assessed
Whole cochlea assessed		Good for anatomical and pathological studies
Vasculature better than osmic		Sensory nervous and vascular structures can be examined at low and high magnification
Good for anatomy		
Disadvantages	*Disadvantages*	*Disadvantages*
Crude	More time-consuming	Vessel lumen artificially injected
Sensorineuroepithelium less well visualized	Bone chips	
Vessel wall lumen artificially injected	Not all parts seen	
Less good for pathology	Deficient contrast	
	Less good for anatomy	



Guinea pig crista ampullaris The rich vascular s visualized through the surface epithelium

rly, few steps are sacred in this ach Fresh solutions carefully prepared f course important, however, variations different steps and the elimination of steps may be suggested as useful modifi is in some experiments For study of the ear vasculature we suggest the procedure mploys Prussian blue only If the vessels be studied in relationship to supporting tures we suggest a light osmic acid ing by injection On the other hand to tigate possible discrete pathological ges we recommend immersion of the lea with opened windows in glutaral de Intracochlear structures may be coun ained with osmic acid after decalcifica with this modification

his method proved useful for study of the nal vasculature and sensorineurepithelium he cochlea as well as the vestibular end in It was also useful in our studies of ex mentally induced changes on both vascula and sensorineurepithelium (induced by electrolytic and mechanical lesions of the ex ternal wall, middle ear pressure changes an influence of noise and/or salicylate, etc) W would further suggest that it may be useful fo studying many other and different experimen tally induced effects on cochlear vasculatur and sensorineurepithelium which give sub stantial cochlear changes observable with ligh and phase contrast microscopy and in magnifi cations ranging from ×10 to ×100 We hav yet to evaluate the usefulness of this metho for electronmicroscopic studies

ZUSAMMENFASSUNG

Eine neue Methode zur Untersuchung der intracoc learen Strukturen sowie der Gefässe und des Se sorineuroepithels wird dargelegt Die Methode i eine Kombination der allgemein angewandten Osmi säuremethode mit gefärbten Häutchenpreparaten u einer Methode bei der Berlinerblau injiziert das Felse bein entkalkt und die Cochlea wiederum Hautchenpreparaten studiert wird Die Kombinati dieser zwei Methoden bewahrt die Vorteile und elimini viele der Nachteile jeder einzelnen Methode Die ve schiedenen Abschnitte der Technik werden detailli

beschrieben und es werden die Variationen in verschiedenen Abschnitten bewertet Ausserdem wird eine photographische Dokumentation der Befunde der neuen Methode im normalen und pathologisch veränderten Innenohr vorgelegt

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A Axelsson M D
Dept of Otolaryngology
University of Goteborg
S-413 45 Goteborg
Sweden

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SPEECH DISCRIMINATION IN QUIET AND IN WHITE NOISE BY PATIENTS WITH PERIPHERAL AND CENTRAL LESIONS

W O Olsen [1] D Noffsinger[2] and S Kurdziel[1]

From the [1]Mayo Clinic and Mayo Foundation Rochester Minn and the [2]Northwestern University Medical School Chicago Ill USA

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Speech-d scrimination scores in quiet and in se (0 dB S/N ratio) were obtained from six groups ts D fferences of 40% or more between scores in d in wh te noise were observed for less than 1% of nal ears tested but were found for 8 0% of ears se trauma 48% of ears with Meniere s disease ears with subsequently surgically confirmed e tumors 14% of ears of patients with multiple and 42% of ears contralateral to the lesion in with temporal lobe damage

persons with sensorineural hearing loss ifficulty in understanding speech during (Simonton & Hedgecock 1953 Palva Ross et al 1965 Liden 1967 Olsen & n 1968 Tillman et al , 1970 Cooper & 1971 Keith & Talis 1972 Shapiro et 72 Jokinen 1973) The most common ation in studies of speech discrimina f monosyllables in the presence of white is the variability among subjects While h-discrimination scores in quiet have uniformly high for persons with normal g scores in the presence of noise have d widely in every investigation Usually ariability in performance has been even r for persons with impaired hearing te this variability differences between s obtained in quiet and in noise have been r for persons with sensorineural (presumably cochlear) hearing losses than for persons with normal hearing

Some researchers have investigated the potential of speech in white noise as a diagnostic indicator of dysfunction at various segments of the auditory nervous system In 1972, Katinsky & Lovrinic reported severe reduction in speech discrimination in white noise from ears of patients who were subsequently found to have 8th nerve tumors

Dayal et al (1966) indicated that speech discrimination in the presence of white noise also can be abnormal in patients with brain stem involvement They reported that some patients with multiple sclerosis had speech discrimination losses in white noise that were not expected on the basis of their pure tone test results Similar findings were described recently by Morales Garcia & Poole (1972) for 10 patients with brain stem tumors and 5 patients with multiple sclerosis and by Noffsinger et al (1972) for a larger sample of patients with multiple sclerosis

Sinha (1959) was the first to report results obtained from the use of monosyllables presented in white noise in the evaluation of patients who had cortical lesions When compared to normal subjects, patients with temporal lobe involvement had decreased performance on speech discrimination in white noise in the ear contralateral to the lesion Patients with cortical lesions that did not affect

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Table I *Speech discrimination study of six groups of subjects*

Group	Sample size	Age years	
		Mean	Range
Normal	75	36 9	13–62
Noise trauma	25	42 2	22–52
Meniere s disease	25	53 1	16–74
8th nerve tumor	21	42 5	17–65
Multiple sclerosis	31	37 8	20–59
Temporal lobe lesion	24	36 ?	18–65

the temporal lobes had scores equal to those of the normal subjects Sinha s observations were supported by the publications of Morales Garcia & Poole (1972) and Heilman et al (1973)

There is no doubt that discrimination of monosyllables can be markedly decreased by lesions in the cochlea as well as by lesions at nearly every segment of the auditory nervous system from the 8th nerve to the temporal lobe However, no previous study has compared speech discrimination performance in quiet and in white noise for patients with cochlear 8th nerve brain stem or cortical lesions utilizing a single test and procedure

PROCEDURE

Routine air-conduction and bone conduction audiometry speech reception threshold and speech discrimination tests were completed individually for all subjects in a double walled test chamber The test equipment was calibrated routinely and maintained to comply with ANSI 1969 specifications (American National Standards Institute 1970) Tape recorded materials were used for all speech tests Speech reception thresholds were determined with spondaic words employing the descending method described by Tillman & Olsen (1973) Speech discrimination was assessed at 40 dB sensation level re the speech reception threshold utilizing Northwestern University Auditory Test No 6 (Tillman & Carhart 1966) in quiet and in white noise at 0 dB signal/noise (S/N) ratio The speech signal and noise were presented via a speech audiometer (Grason Stadler 162) The op site ear was masked for bone-conduc testing and for the air-conduction pure t and speech tests as needed A separate n source was used to mask the opposite when this was necessary during the speec white noise test

SUBJECTS (Table I)

A group of 75 subjects (150 ears) with ne apparent disease nor significant hearing l was tested to provide normative data aga which the performance of the patients peripheral auditory disease or cen nervous system involvement were compa These normal subjects had pure tone thr olds from 125 to 8000 Hz, no poorer than 2 HL (re ANSI 1969) at any frequency pure tone averages (500 to 2000 Hz) speech reception thresholds of 25 dB HL ANSI 1969) or better bilaterally their spe discrimination scores were 90% or bette quiet at 40 dB SL The mean age for t subjects was 36 9 years and the ages ran from 13 to 62 years None was a sophisti or practiced listener

The other groups consisted of pat ents medically diagnosed hearing losses due noise trauma Meniere s disease or 8th n tumors (subsequently surgically confir and patients with lesions of the central n ous system Patients with central nerv system involvement were separated into groups patients with multiple scleros patients who had documented temporal involvement due to cerebrovascular acci or who had undergone neurosurgery for s from intractable seizures

Twenty five patients had noise trau Their pure tone averages and spe reception thresholds were within normal (25 dB HL or better) but each had characteristic audiometric configuration sociated with noise induced or acou trauma hearing impairments that is a h frequency notch of reduced sensitivity was deepest at 3000 4000 or 6000 Hz w

I *Pure tone and speech results for six groups of subjects*

	No of ears	Pure tone (dB HL)		Speech reception threshold (dB HL)		Speech discrimination (%)					
						Quiet		White noise		Difference*	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
	150	6 5	−7 to 21	3 8	−5 to 21	98 2	92 to 100	73 8	56 to 94	24 4	6 to 44
uma	50	11 0	0 to 23	6 0	−5 to 18	97 2	90 to 100	69 3	52 to 88	27 9	4 to 44
d sease	25	42 1	27 to 62	38 1	15 to 64	85 4	60 to 100	44 6	0 to 72	40 8	18 to 82
tumor	21	25 9	10 to 48	22 2	−7 to 45	84 0	56 to 100	36 8	0 to 74	47 2	20 to 92
sclerosis	50	8 4	−2 to 18	5 0	−6 to 18	97 6	92 to 100	70 7	36 to 92	26 9	6 to 54
obe	24	11 9	0 to 32	10 4	0 to 30	96 3	84 to 100	53 2	8 to 78	43 1	22 to 84

nus white noise scores

hearing sensitivity at 8000 Hz This was common to both ears of all these ts hence, there were 50 ears in the rauma sample Speech discrimination in as 90% or better for all of them at 40 dB

nty five patients had a medical diag of unilateral Meniere's disease, this consisted of 25 ears Various degrees low frequency hearing loss associated 'eniere s disease were present at the 'our testing as reflected by the range of ure tone averages (27 to 62 dB HL) and ı-discrimination scores in quiet (60 to (Table II)

nty-one patients subsequently found to nilateral 8th nerve tumors (surgically ed) were tested (Only the results for impaired ears are given in Table II) ears demonstrated hearing sensitivity ıg from normal to mild hearing impair for speech and speech discrimination in anging from 56 to 100%

rty-one patients had confirmed multiple sis The ears of these patients were ıd to have 125 to 8000 Hz pure tone ls within normal limits (25 dB HL or ı a speech reception threshold of 25 dB tter and speech discrimination of 90% or r These criteria were imposed in order to ss possible influences of central nervous m lesions associated with multiple is on speech discrimination in white noise without the contaminating effects of a loss in hearing sensitivity attributable to other involvements Both ears of 19 of the patients and one ear of each of the remaining 12 patients met these criteria

Of 24 patients with temporal lobe lesions, three had had hemispherectomies when they were young in order to overcome serious convulsive disorders The left cortical hemisphere had been removed in one patient, and a right hemispherectomy had been performed in the other two Eleven patients had undergone partial temporal lobectomies (six left, five right) to ocvercome siezures The other 10 patients had suffered cerebrovascular accidents that affected the temporal lobe The damage had occurred in the left temporal lobe in six patients and in the right temporal lobe in four The ears contralateral to the damaged hemisphere were included in this sample These ears were characterized by essentially normal speech reception thresholds and good speech discrimination in quiet (84 to 100%) (Table II)

Each subject tested in this project was capable of communicating with the audiologist and of understanding and following test instructions All were able to respond reliably to the various auditory tests administered

RESULTS

None of the 150 ears in the normal hearing group had pure tone averages or speech

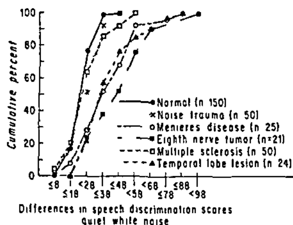


Fig 1 Cumulative frequency distribution of speech-discrimination scores (quiet minus white noise) for six groups of subjects, *n*, number of ears in each group

reception thresholds poorer than 21 dB HL, and all had speech-discrimination scores in quiet of 92% or better (Table II) When the monosyllables were mixed with white noise at a S/N ratio of 0 dB, the average socre was almost 74%, the range was from 56 to 94% The mean difference between scores in quiet and in white noise was about 24% and ranged from 6 to 44%

The mean score in quiet for the 50 ears in the group with noise trauma was similar to that of the normal group Speech discrimination in noise, however, was about 4% poorer on the average than for the normal group The range of performance in noise was about the same for both groups

As expected, the 25 patients with unilateral Meniere's disease had average thresholds and speech-discrimination scores in quiet that were poorer than those of the group with trauma In addition, their breakdown in formance in white noise was also gr 40 8% as compared to 27 9% for the with noise trauma The range of differ (quiet minus white noise scores) was larg the group with Meniere's disease (18 to 8

Even though the 21 ears with 8th tumors generally had better hearing sensi than did the ears with Meniere s diseas had speech discrimination scores in quie were equal to those of the group Meniere's disease, their average in white noise was about 8% poorer

The performance of the group with mu sclerosis was similar to that of the n hearing group and the group with noise ma However, the scores in white obtained for some of the patients with mu sclerosis were poorer than scores for a the normal subjects, although both group normal hearing sensitivity and speech crimination in quiet

The group with temporal lobe lesions essentially normal sensitivity and speech crimination in quiet for the ears contral to the cortical lesion Only one patient hearing sensitivity poorer than 25 dB HL speech discrimination below 90% in q However, the average difference betw their scores in quiet and in white noise w large as that observed for the group Meniere's disease and the group with nerve tumors although the group temporal lobe lesions had better hea sensitivity and speech discrimination in qu

Table III *Incidence (in per cent) of differences in speech-discrimination scores for six grou subjects*

Group	Difference scores (quiet minus white noise scores) 0–8	10–18	20–28	30–38	40–48	50–58	60–68	70–78	80–88	90-
Normal	0 7	16 0	60 6	22 0	0 7					
Noise trauma	2 0	10 0	40 0	40 0	8 0					
Meniere s disease		8 0	20 0	24 0	16 0	24 0		4 0	4 0	
8th nerve tumor			23 8	14 3	14 3	23 8	14 3	4 8		4 8
Multiple sclerosis	4 0	16 0	44 0	22 0	6 0	8 0				
Temporal lobe lesion			22 8	35 0	19 2	7 7	3 8	7 7	3 8	

IV *Pure-tone and speech results for roups of patients with cortical lesions*[a]

	Group			
	Temporal lobectomy (11 patients)		Cerebrovascular accident (10 patients)	
	Mean	Range	Mean	Range
ne (dB HL)	6 9	0– 20	19 7	11– 21
reception hold (dB HL)	6 8	0– 15	16 2	9– 30
(%)	99 2	92–100	93 2	84–100
noise (%)	68 9	62– 78	35 4	8– 54
nce (%)[b]	30 3	22– 38	57 8	36– 84

ontralateral to lesion
t minus white noise test scores

st of the normal ears had scores in noise were 20 to 28% poorer than their rmance in quiet (Fig 1 and Table III) of the remainder achieved scores that 10 to 18% or 30 to 38% poorer Only two varied from this pattern, one attaining a that was reduced from the score in quiet % and one by 44% These data indicate a difference of 40% or more between h-discrimination scores in quiet and in noise from these test materials is un for persons with normal hearing There differences of 40% or greater were con -d abnormal for the purposes of this

ie distribution of quiet white noise differ- s for the group with noise trauma was ar to that of the normal group, but there a slight skewing of the distribution to the (Fig 1) There were four ears (8%) in this p whose scores were reduced by 40% or e

he incidence of marked reduction in ch understanding in white noise was con- rably greater for the group with Meniere s ase about one-half of the patients in this p attained scores in white noise that were uced by 40% or more from their scores in t The distribution for the group with niere s disease was different from that of the normal group or the group with noise trauma

Quiet-white noise differences exceeding 38% was most frequent in the group with 8th nerve tumors The decrease in speech discrimination performance in white noise was most commonly from 40 to 68%, one patient had a score of 92% in quiet but was unable to understand even one monosyllable in white noise at a 0 dB S/N ratio The cumulative distribution curve for this group paralleled the curve for the group with Meniere's disease but was shifted somewhat to the right throughout (Fig 1)

While most patients with multiple sclerosis did not have inordinate difficulties in understanding speech in white noise, seven (14%) had performances decreased by 40% or more by the addition of white noise Decreases this large were unilateral for five patients and bilateral for one patient This incidence of excess breakdown in speech discrimination in white noise is striking when one considers that each of these subjects was required to have normal hearing sensitivity and speech discrimination in quiet in order to be included in the study The lower part of the cumulative distribution curve for this group followed or fell between the curves for the normal group and the group with trauma but then fell below these groups in the upper portion (Fig 1)

A higher incidence of excess decrease in

Table V *Incidence (in percent) of differences in speech discrimination scores for two groups of patients with cortical lesions*

	Group	
Difference scores (quiet minus white noise scores)	Temporal lobectomy (11 patients)	Cerebrovascular accident (10 patients)
20–28	45 5	0 0
30–38	54 5	10 0
40–48	0 0	30 0
50–58	0 0	20 0
60–68	0 0	10 0
70–78	0 0	20 0
80–88	0 0	10 0

understanding speech in white noise was observed for the contralateral ears of patients with temporal lobe lesions Approximately two-fifths of these patients had decreases of 40% or more in speech discrimination performance when white noise was mixed with the speech signal Curiously, the cumulative distribution curve for this group paralleled most closely the curve for the group with Meniere's disease in spite of no loss in hearing sensitivity for the group of patients with temporal lobe lesions For all 24 of these patients, scores in white noise in the ipsilateral ear were reduced no more than 38% from their optimal performance in quiet, the mean decrease was 28 4%, and the range was 16 to 38%

DISCUSSION

Our data support the rather marked variability in the performance of subjects with normal hearing in speech discrimination in noise that has been noted by others The range of differences in quiet white noise scores obtained in our study was similar to that reported by Keith & Talis (1972) but was somewhat less than that shown by Morales Garcia & Poole (1972) for similar 0 dB S/N ratio conditions The rather sharp delineation in performance observed in our study (with only one normal ear yielding a difference greater than 38% between scores in quiet and in white noise) is encouraging and suggests that a difference of 40% or greater can be considered abnormal when using the test materials utilized in this study However, clinicians who wish to utilize a speech in noise test must gather normative data with the specific test materials they use Some investigators have employed more favorable S/N ratios in order to obtain scores sufficiently above 0% to observe meaningful differences in performance Furthermore consideration of the range and distribution of performance by a group of normals is most important if a test is to be utilized clinically

The data obtained in our study for the group with *noise trauma* (with the associated h *frequency loss) and the group with Meni* disease (generally with low frequency or hearing impairments) are in agreement the findings of Ross et al (1965) and Kei Talis (1972) They noted that speech-d sc nation performance in white noise was severely affected by low frequency sen neural hearing impairments The results their studies, however, cannot be directly to findings from our study bec they did not report the cause of the he impairments Nevertheless there is agree among the studies of Ross et al (1965) K & Talis (1972) and the present study low frequency hearing losses generally crease speech discrimination performan noise more severely than do high frequ losses when the impairments are attribu (presumably) to cochlear involvements

As suggested by Katinsky & Lo (1972), patients with 8th nerve tumors have excess reduction in speech discrim tion in white noise The data from our st however, indicate that considerable ca must be attached to such an assertion in our study eight patients with 8th n tumors had scores in quiet and in white n that differed by less than 40% Second data indicates that other auditory inv ments also can result in a marked reductio speech-discrimination performance in w noise

Observation of differences of 40% or in white noise via the contralateral ear has been documented earlier (Sinha ple sclerosis supports previous report Dayal et al (1966) Morales Garcia & (1972) and Noffsinger et al (1972) In present study six of the 31 patients multiple sclerosis revealed excess reductio speech discrimination in white noise unilaterally and one bilaterally) in spit normal hearing sensitivity for all aud om test frequencies and excellent speech crimination in quiet Neurologic symptom these six patients varied within the ce

ous system, and there was no obvious onship between the breakdown in white and sidedness, bilaterality, or inferred of lesion Morales Garcia & Poole (1972) similar conclusions

e difficulty encountered by patients with oral lobe lesions in understanding speech hite noise via the contralateral ear also been documented earlier (Sinha 1959, ales Garcia & Poole, 1972, Heilman et al ,) However, consideration of data ob d from the patients with temporal lobe ns in our study revealed two distinct ps

rformance of the 11 patients who had rgone partial temporal lobectomy for in able seizures was within normal limits les IV and V) Of the 10 patients who cerebrovascular accidents, only one (left brovascular accident) showed normal ormance on the speech in white noise test other nine patients had greater difficulty this task showing differences of 42 to between their scores in quiet and in white e (Table IV) Of three patients with ispherectomy two revealed excess break n in speech discrimination in white noise right one left) whereas the third (right ispherectomy) did not

ndings for the patients with temporal ctomy contrast with the results of Sinha 9) and of Heilman et al (1973) who noted reased performance for the ear contra ral to the lesion compared to performance the ipsilateral ear Such differences be en ears were not observed for the patients h temporal lobectomy in our study in that mean decrease for the ipsilateral ear was 3% compared to 30 4% for the contra ral ear of these patients This result was ained even though as much as 52 mm of the erior portion of the left tempor il lobe or 70 of the anterior portion of the right nporal lobe were removed in individual tients

From these results it would appear that the mage either involved portions other than the anterior segment or involved more than the anterior portion of the temporal lobe for the patients with cerebrovascular accident This statement is based on the observation that all but one of these patients demonstrated excess difficulty on the speech in the white noise test Of course, for the patients with hemispherec tomy, the entire temporal lobe had been re- moved, two of these revealed differences of 46 and 48% between their scores in quiet and in white noise, but one scored only 32% poorer in noise than in quiet

The difference in performance via the contralateral and ipsilateral ears is an im portant consideration in patients with cortical lesions The observation that, when excess difficulty on the speech in white noise was found it was always for the ear contralateral to the lesion regardless of whether the lesion was in the right or in the left temporal lobe, is in agreement with the findings of the previ ously cited investigators Such findings indi cate that the right temporal lobe is also in volved in processing or transmitting (or both) speech signals even though the left temporal lobe is generally considered to be the domi- nant language center in man These comments are consistent with the conclusions reached by Sparks et al (1970) and Berlin et al (1972) based on their findings from dichotic speech tests of patients with temporal lobe lesions It also demonstrates the dominance of the contralateral auditory tract in carrying infor mation to the temporal lobe

From the preceding discussion it is appar ent that lesions at any point in the auditory system from the cochlea through the temporal lobe can result in marked reduction in speech discrimination in white noise In patients with cochlear involvements low frequency hearing impairments apparently disturb speech dis crimination in white noise more than do coch lear lesions resulting in high frequency losses However large differences between speech discrimination scores attained in quiet and in white noise in conjunction with normal hearing sensitivity or even a predominantly high

frequency hearing loss probably reflect neural involvement of some portion of the auditory system Thus, results from speech in the white noise test may have some clinical use fulness in revealing abnormalities in auditory function but not in suggesting a particular site of involvement as being responsible for the dysfunction

ZUSAMMENFASSUNG

Die Ergebnisse der Sprachdiskrimination im Stillen und in Weissem Larm (Verhaltnis 0 dB S/N) von sechs Ver suchsgruppen wurden untersucht Bei den Ergebnissen im Stillen und im weissen Larm wurden Unterschiede von 40 % oder mehr bei weniger als 1 % der gepruften norma len Ohren beobachtet Diese Unterschiede wurden aber bei folgenden Gruppen festgestellt bei 8 0 % der Ohren mit Lärm–Trauma bei 48 % der Ohren mit Meniereschem Syndrom bei 62 % der Ohren mit nachtraglich aufgrund chirurgischer Eingriffe festgestellten VIII Nerv Tumo-ren bei 14 % der Ohren von Patienten mit multipler Sklerose und bei 42 % der den Temporallappenver letzungen gegenuberliegenden Ohren von gehirnge schadigten Patienten

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W O Olsen Ph D
Mayo Clinic
Mayo Foundation
Rochester Minn 55901 USA

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UNDISTORTED AND FILTERED SPEECH AUDIOMETRY IN CHILDREN WITH NORMAL HEARING

A Palva and K Jokinen

From the Department of Otolaryngology University of Oulu Oulu Finland

(Received March 1 1975)

act Undistorted and filtered speech tests were car *out in 140* test subjects with normal hearing acuity ng in age from 4 to 19 years Routine adult word lists used in all tests The intelligibility of undistorted h rose markedly in the age groups from 4 to 9 years orrespondingly in the filtered speech test up to 11 taking the group 16–19 years as basis of reference rd ng to these results a speech discrimination test can ide in childhood with the same methods as in adults if orms are taken into account In the filtered speech test naural test result and the monaural discrimination y of the left ear were significantly better than the ural test results for the right ear up to the age of 12–13

ch discrimination tests can be carried out re school age by using the ordinary tests ared for adults utilizing the standards in rent age groups Even distorted speech have been utilized in children (Boothroyd, l) using low pass and high pass filtering ech discrimination scores in children were ceably lower than those of adults High filtering had a more pronounced effect l low pass filtering on the scores in dren Maximal discrimination of normal ring children increased significantly be en the ages of 6 and 9½ years (Boothroyd, 8) Siegenthaler (1969) supposed that the litory functions have stabilized at the adult l at the age of 12 years and in most cases n at 8 years

study was supported in part by a grant from the e Medical Research Council Helsinki Finland

To get a good reference score for speech audiometry in children using the same methods as for adults, we performed undistorted and filtered speech discrimination tests in a group of normal children

MATERIAL AND METHOD

The study consisted of 140 test subjects, 78 girls and 62 boys, ranging in age from 4 to 19 years There were 20 subjects in each of the age groups of 4–5 years, 6–7 years, 8–9 years, 10–11 years, 12–13 years, 14–15 years and 16–19 years

Routine clinical otorhinolaryngological examination was made in each case None of the test subjects had any ear diseases

Pure tone threshold measurements by air and bone conduction were made at 125, 250, 500, 1000, 2000 4000, 6000 and 8000 Hz with a Madsen Model OB 60 audiometer equipped with Beltone TDH 39 earphones Calibration of the audiometer was made according to the ISO standard

Undistorted speech audiometry was carried out by the method of T Palva (1952), measuring the speech reception threshold (SRT) and the discrimination of speech 30 dB above the SRT The same vocabulary was used as in routine adult tests A Madsen Model SU 20 speech unit was connected to the system used in pure tone audiometry

Table I *Average pure tone thresholds in different age groups*

Age group		Frequency (Hz) 125 Mean	S D	250 Mean	S D	500 Mean	S D	1000 Mean	S D
4–5	right	14 0	5 0	11 2	5 1	8 7*	3 9	8 5**	2 9
	left	13 7	4 5	11 0	4 8	9 0*	4 5	8 0*	4 1
6–7	right	12 2	4 7	10 0	5 1	7 7	4 4	6 2	4 2
	left	11 7	5 4	9 0	5 8	7 2	4 1	4 7	4 4
16–19	right	11 2	3 6	8 7	2 7	5 5	2 8	4 2	2 4
	left	9 5	2 2	8 0	2 5	5 7	2 9	4 5	3 2

Differences were calculated relative to the oldest age group
Significance level $p \leq 0\ 01 = *$ $p \leq 0\ 001 = **$

The filtered speech test was carried out by the method of A Palva (1965) Two bands of speech, 480–720 Hz and 1 800–2 400 Hz, were used Each band alone gives the same discrimination of about 15–20% When the bands are presented together monaurally or binaurally, each band to either ear, the level of discrimination rises to about 80% in normal young people of 20–25 years

Word lists are used so that the test words are presented to the subject automatically in the following order The first word is given to the right ear on both bands, the second to the left ear on both bands, the third word binaurally one band to the right ear and the other to the left, the fourth word in a similar manner to the first and so on Each test, on the right ear, on the left ear and binaurally, is carried out using 90 words One sensation level of 50 dB is used Cross hearing is eliminated by using insert type earphones The test yields three discrimination percentages, one for the right for the left ear and a binaural one

The statistical analyses were perf the Computer Centre of the University The averages and their standard de were calculated Comparison of two were made by applying the Student s difference is termed significant if responding value of probability (p) is

RESULTS

The pure tone thresholds of the two age groups and the oldest group are Table I The averages for both ears we lated separately in each group and frequency No significant difference the right and left ear was obtained frequenc group 4– 1 000 and 2 000 Hz and the group 6–

Table II *Discrimination scores in undistorted speech audiometry*

	Age group 4–5 Mean	S D	6–7 Mean	S D	8–9 Mean	S D	10–11 Mean	S D	12–13 Mean	S D	14–15 Mean	S D	16- Me
Right	83 3**	6 9	89 1**	6 1	90 1**	5 2	95 2	5 0	96 5	3 7	96 4	5 5	98
Left	82 8**	7 4	88 5**	6 6	90 6**	5 9	92 6	5 0	97 2	3 0	97 1	3 9	96

Differences were calculated relative to the oldest age group
Significance level $p \leq 0\ 01 = *$ $p \leq 0\ 001 = **$

S D	6000		8 000	
	Mean	S D	Mean	S D
3 4	8 2	4 4	10 2	7 3
5 0	9 7	6 6	11 2	8 9
5 1	9 7	5 2	8 5	5 1
4 7	8 0	5 7	8 5	6 5
3 9	7 5	8 8	10 0	8 4
4 5	8 2	7 7	8 5	8 7

Hz from the oldest group The pure tone holds in all other age groups were the same r the oldest group

sults of the undistorted speech test are n in Table II Discrimination of undis d speech in the three youngest age groups significantly poorer than in the oldest but the loss of discrimination was only % There was no significant difference een the right and the left ear in any of the roups

ble III and Fig 1 shows the results of the d speech test Discrimination was found e gradually with increase in age The three gest age groups differed significantly from dest group as regards all three test objects he difference in discrimination between roups 10–11 years and 10–19 years was icant in the right ear and in the binaural In the youngest age group the discrimina- values were about 40 % lower than in the t group Individual variations in results slightly larger in the three youngest age ps

The differences in discrimination scores for left-ear, right ear and binaural hearing were calculated in each individual case and the results treated statistically (Table IV) Discrimination was significantly better in the left ear than in the right in the groups 4–5 years, 6–7 years, 10–11 years and 12–13 years Binaural discrimination was significantly better than the figure for the right ear in the groups 4–5 years and 10–11 years Discrimination in the left ear did not differ significantly from the binaural result in any one of the groups

Differences in discrimination between the left and right ear in the monaural test are given in Fig 2 Asymmetry in discrimination score exceeding 15 % was found in the groups 10–11 years in 2 cases (10 %), 12–13 years in 1 case (5 %), and 16–19 years in 2 cases (10 %) In the oldest age group the difference was less than 5 % in 70 % of cases, while in the three youngest groups the corresponding figures were 35 %, 40 % and 60 % Binaural discrimination was in no case more than 10 % inferior to the result for the poorer ear in the monaural test Thus a positive binaural test was obtained in none of the groups

DISCUSSION

Our results as regards pure tone thresholds agree with those reported in earlier studies (Eagles et al , 1963, Siegenthaler, 1969, Fior 1972) There was a significant increase in hearing acuity over the age range 4–7 years while from 8 years upwards none of the groups differed significantly from the oldest age group

III *Discrimination scores in filtered speech test*

	Age group													
	4–5		6–7		8–9		10–11		12–13		14–15		16–19	
	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D
	41 3**	13 2	54 8**	10 0	66 4**	14 0	73 0*	7 9	78 8	11 0	82 9	7 7	82 4	10 8
	46 7**	10 9	58 9**	10 4	68 6**	11 2	79 2	7 6	83 9	9 1	85 7	6 8	85 4	11 3
ral	46 3**	12 5	56 5**	11 4	68 6**	13 8	78 1*	6 6	81 9	11 3	84 5	7 2	85 2	8 3

ences were calculated relative to the oldest age group
ficance level $p \leq 0\ 01 =$ * $p \leq 0\ 001 =$ **

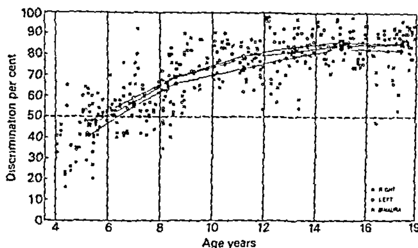


Fig 1 Effect of age on filtered speech test

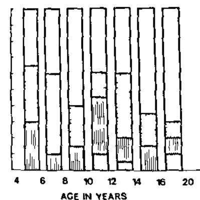
For speech audiometric studies we used the same word lists as in adults Discrimination scores in the nondistorted speech test increased significantly in the age range from 4 to 9 years compared with the oldest age group In the age group 4–5 years the discrimination score was about 15% lower than in the oldest age group whereas in the group 10–11 years the score was only 3% lower

According to Boothroyd (1970) the relatively lower scores of younger normally hearing children may be due to the fact that the child may not have a complete set of phonemic categories or his vocabulary may not include all the test words Furthermore the ability to carry out the decision process may be imperfectly developed and developmental articulation difficulties may lower the younger child's score On the other hand it is necessary to distinguish between passive and active vocabul children The child, like the adult re appropriately to many words that he d use The number of words that the child and uses will depend on the number of w has been exposed to and therefore a socioeconomic factors By the age of 7 the subjects studied by Templin (1957) mean estimated passive vocabulary of 20 000 words, with a standard deviat about 10 000 On the basis of these da our own experience, the same test w for adults can be used also for childre the age of 4–5 years, when related norms for children In younger ch special tests must be used (Siegenthaler 1954, Sortini 1964)

Table IV *Differences between monaural and binaural discrimination scores in filtered s test*

	Age group 4–5		6–7		8–9		10–11
	Mean	S D	Mean	S D	Mean	S D	Mean
Left ear superior to right	5 4**	5 9	4 3**	4 6	2 2	5 8	6
Binaural test superior to right ear test	5 0*	6 1	1 5	7 6	2 2	5 3	5 6*
Binaural test superior to left-ear test	–0 4	–7 2	–2 4	7 9	0 0	7 8	–1 1

Differences were calculated separately for each individual case correlated to the test object concerned
Significance level $p \leqslant 0\ 01 = *$ $p \leqslant 0\ 001 = **$



Asymmetry in the monaural test at various ages

oup In the youngest group the discrimi-
n scores were poor, about 40 % lower than oldest group

cording to Boothroyd (1970), high pass ng has a greater effect on children's s than low-pass filtering In his opinion oorer performance of children must be y due to the increased difficulty of the test bulary However, this cannot be the only r since the drop in score depends on the of filtering

eech audiometry sensitized by frequency tion is mainly used for the detection of al hearing disorders (Bocca et al , 1954, ker 1958 Jerger, 1960, Korsan Bengtsen, Palva & Jokinen, 1975) In addition age ed degenerative changes have an effect on mination (Palva & Jokinen, 1970)

he binaural test result and the monaural discrimination ability of the left ear were significantly better than the monaural test results for the right ear up to the age of 12–13 years Anatomical development, notably myelinization, is incomplete in the first years of life Maturation of hearing abilities is a gradual process which reaches its maximum at the age of 12–13 years (Siegenthaler, 1969, Fior, 1972) Since the developmental stage in the normal-hearing child is the same in both ears, an asymmetric test result may indicate a functional asymmetry in the auditory system at those levels where asymmetry can appear physiologically The results may be due to cerebral dominance in hearing (Penfield & Roberts, 1959, Milner et al , 1964, Shankweiler, 1966, Palva & Jokinen, 1970) In normal young adults the compensatory mechanism of the centrencephalic system may exclude the effect of cerebral dominance in the filtered speech test

Matzker (1958) found positive binaural test results in 34 % of the children under 14 years of age The difference between his results and ours is probably caused by differences in testing technique

The fact that the binaural discrimination did not significantly differ from the discrimination of the better ear shows that the binaural synthesis of two different frequency bands is not affected in children

	14–15		16–19	
S D	Mean	S D	Mean	S D
8 0	2 8	5 4	4 4	8 8
6 4	1 6	4 5	2 3	5 0
8 5	−1 2	5 9	−0 1	9 3

ZUSAMMENFASSUNG

140 Probanden im Alter von 4 bis 19 Jahren mit normalem Horvermögen wurden Sprachtests mit normaler und mit filtrierter Sprache unterzogen Alle Versuche wurden mit den von Erwachsenen benutzten Wörterbuchern durchgefuhrt Nimmt man die Gruppe der 16–19jahrigen als Vergleichsmasstab so nahm die Fähigkeit normale Sprache zu verstehen bei den 4–9jahrigen mit dem Alter deutlich zu und bei filtrierter Sprache entsprechend in der Gruppe bis zu 11 Jahren Diese Ergebnisse zeigen dass Hordiskriminationstests schon im Kindesalter durchgeführt werden konnen und zwar—werden die Normen eingehalten—mit denselben Methoden wie bei Erwachsenen Bei filtrierten Tests waren bis zu 12–13 Jahren hin die binauralen Ergebnisse und die monaurale Diskriminationsfähigkeit des linken Ohres erheblich besser als die monauralen Ergebnisse mit dem rechten Ohr

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A. Palva, M.D.
Dept. of Otolaryngology
University of Oulu
SF 90220 Oulu 22
Finland

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OTOTOXICITY OF TOPICALLY APPLIED GENTAMICIN USING ı STATISTICAL ANALYSIS OF ELECTROPHYSIOLOGICAL MEASUREMENT

T Morizono and B M Johnstone

From the ENT Department The Sir Charles Gairdner Hospital Perth Medical Centre Shenton Park and the Physiology Department University of Western Australia Nedlands Western Australia

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ıct Ototoxicity of topically applied gentamicin was in guinea pigs 0 3% gentamicin was instilled in de of the middle ear cavity and Ringer s solution was ed in the other side and the difference in the microphonics measured with the round window ode was analysed statistically Instillation of Ring ion in the middle ear cavity for 1 day did not any significant sensorineural hearing loss but on rd day of instillation significantly reduced responses observed compared with the responses from non ed ears followed by partial recovery starting on the When gentamicin 0 3% was instilled into the le ear cavity significant deafness occurred 24 hours and highly significant deafness on the 2nd and 3rd The usage of gentamicin ear drops of the current ıla should be discouraged until a better formula is ıded

› of hearing impairment following tamicin using an electrophysiological hod were done by Lundquist & Wersall ›ı) Soda et al (1968), Hawkins et al (1969) Brummett et al (1972) using guinea pigs the drug was administered systemically in very high doses, viz 100 mg per kg for days (Lundquist) 100 mg and 200 mg per for more than 19 days (Hawkins) and 50–) mg for 4 weeks (Brummett)

The effects of topically applied gentamicin studied only by Wersall et al (1969 ıı Webster et al (1970 1971) and Stupp et (1973) who have made histological studies Since there is no experimental work on the of topically applied gentamicin on the ear audiogram this work was undertaken find out if clinically prescribed gentamicin drops can cause deafness

work was supported by the N H & M R C Re ı Grant from the Commonwealth of Australia

METHOD AND PROCEDURE

A total of 85 guinea pigs of mixed sexes and of tortoise shell colour were employed in this study Of these animals, 3 had middle ear infection, 2 had bilateral sensorineural deaf ness and 4 died of anaesthetic accidents The guinea pigs were obtained from our central animal house where they have been interbred for the last 10 years The body weight of the animals used was between 250 and 350 grams and all showed normal Preyer's reflex indicating detectable hearing

Under local anaesthesia with 1% Xylocaine, an incision, 5 mm in length, was made at the retro-auricular region on the posterolateral part of the bulla Two pin holes were made on the tympanic bulla, one for applying the test solution and the other for equilibrating the pressure in the middle ear cavity during the instillation of the test solution The middle ear cavity was filled up with ear drops which were diluted with Ringer's through a pin hole using a 26 gauge needle, the middle ear cavity of the other side was filled with Ringer s as a control, and the animal was returned to the cage for a few days until required

To measure the cochlear microphonic response, the animal was anaesthetised with 30 mg per body weight of Nembutal A tracheostomy was performed and the animal was respirated with air using a small animal res pirator A muscle relaxant, Alloferin, was given from time to time, when necessary The *auditory bulla was exposed lengthen*

Table I Cochlear microphonics from the control group (in dB scale) at 70 dB SPL

Frequency (Hz)	N	Mean (dB)	S D (dB)	For new observation P=5%	P=1%
125	33	51 4	5 0	10 0	13 6
250	35	60 2	5 6	11 3	15 3
500	35	66 3	4 4	8 8	11 9
1 000	35	69 1	3 4	6 8	9 1
2 000	35	64 4	3 8	7 8	10 5
4 000	35	65 0	5 0	10 2	13 6
8 000	35	59 9	4 4	8 9	11 9
Average			4 5±0 8	9 1±1 5	12 3±2 1

In the tables, 0dB is equivalent to 0 1 μV hence 60 dB is equivalent to 100 μV

former incision, and a 3 mm hole was drilled in its posterior lateral surface Using an operating microscope, the round window niche was located The animal was positioned so that the *round window was in a horizontal plane*

The test solution as well as all exudates was thoroughly removed using a small suction tube and a fine wick made of tissue paper The corner of the middle ear cavity including the round window niche and tympanic membrane was carefully examined prior to the measurement of the responses A silver wire electrode insulated except for the rounded tip, was placed on the round window membrane and an Ag–AgCl electrode was placed at the corner of the incision wound for the reference electrode

The sound stimulus generated by an oscillator and loudspeaker was introduced into the external auditory canal through a tube and adaptor and the intensity of the sound was measured at the ear drum level using the same adaptor with a calibrated probe tube microphone An octave band filter was used to obtain a better signal noise ratio

Input–output function curves of the cochlear microphonics for seven frequencies viz 125, 250, 500, 1000, 2000 4000 and 8000 Hz were made from all the animals except for those which showed no measurable response at 70 or 80 dB SPL For these animals, the maximum response and the sound pressure required for this response, were measured i order to distinguish sensorineural deafn from conductive deafness

RESULTS

(1) *Cochlear microphonic responses from non-treated guinea pigs*

As a control, 35 guinea pigs were used in th study The R M S voltages of cochle microphonics under various frequencies a under various sound pressures were mea ured using a B & K microphone amplifier T voltages were converted to a dB scale 0 being set to 0 1 μV r m s Use of a dB sca facilitated easy comparison and analysis of t results, and the conversion of this dB chan to the corresponding voltage can easily made using a semi log chart

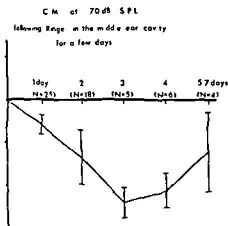
As shown in Table I, the amplitude of t cochlear microphonic responses at a sou pressure level of 70 dB SPL was maximum 1 kHz and this frequency gave the most st output among the different animals while t maximum variations of the observed volta were at 250 Hz

The mean output for the 1 kHz at 70 dB S was 69 1 dB (re 0 1 μV) which is equivalen 280 μV Since its standard deviation (hereaf S D will be used) was 3 35 dB accordin the Student s two tailed *t* test, new obser

Table II Cochlear microphonics at 70 dB S following Ringer instillation in the middle cavity for 24 hours

Frequency (Hz)	N	Mean (dB)	S D (dB)	For new observation P=5%	P 1%
125	20	50 4	5 2	10 8	14 8
250	25	57 2	6 6	13 5	18 3
500	25	64 2	5 6	11 6	15 7
1 000	25	66 0	4 1	8 5	11 5
2 000	25	59 9	4 3	8 8	11 9
4 000	25	61 6	6 4	13 7	17 8
8 000	24	58 5	5 2	10 7	14 6
Average			5 3±0 9	11 0±1 9	14 9

The measurements were carried out after the middle cavity was carefully cleaned out see text



Mean hearing loss in 7 frequencies are averaged eir S D s are expressed with vertical bars on either f the line The responses from non treated ears are is the control

of 6 8 dB smaller than the mean (62 3 130 μV) and 9 1 dB smaller than the mean dB=100 μV) could be decided to be ficant hearing loss of $P=5\%$ and $P=1\%$ ctively

ing the same procedure, the significance of the hearing loss at each frequency was mined statistically

ochlear microphonic responses following er instillation into middle ear cavity

malian Ringer s solution (pH adjusted to was used for instillation into the middle avity as a control and as the solution to e test solutions

ne responses 24 hours after instillation of Ringer are summarised in Table II The onses from the non treated group (Table I from this group (Table II) have essen- y the same pattern When the difference of ns in Tables I and II was compared at each uency the Ringer treated group showed es 2 0±1 3 dB lower than those of the treated group, but the difference was istically not significant

he question arises here as to what would pen to inner ear function if the Ringer was in the middle ear cavity for a much longer iod Our study revealed that the Ringer caused some sensorineural hearing loss and considerable recovery followed within a week The loss of hearing for each frequency was averaged and its S D was calculated (Fig 1)

The reduction of the responses reached a maximum on the 3rd day, and recovery occurred from the 4th day. The most marked reduction of the responses occurred at 4 kHz and the recovery of the responses at that frequency appeared to be slow

(3) *Results with gentamicin otic solution, 0 3% in middle ear cavity*

The following procedure was carried out

Firstly, using Table II, the animals which showed significantly lower responses from the control side at any frequencies were omitted from the present study This procedure was necessary in order to exclude the influence of any preceding inner ear disease or of insufficient ventilation of the animal due to undetected respiratory obstruction which may have caused a lower response

Secondly, the differences between the left and right ears were analysed statistically using the S D 's of Table II and the two tailed *t* test The procedure seemed to give us a more precise picture regarding the hearing loss caused by the test drugs contained in the Ringer because the absolute response as well as the change of the response due to Ringer varies considerably from animal to animal, hence the comparison of the absolute voltages from the ears which had the test solution with

Table III *Deafness following gentamicin otic (0 3% Full strength) at each frequency*

Animal	Duration (Day)	Degree of hearing loss						
		125	25	5	1	2	4	8 kHz
1	1D	–	±	–	–	±	–	
2	1D		±	±	±	+	±	±
3	1D	±	±	+	±	+	+	+
4	1D	–	–	–	–		–	±
5	2D	++	++	+	++	++	±	++
6	3D	–	–	–	±	+	–	+
7	3D	++	++	++	++	++	++	++

±, $30\% \geq P > 5\%$ +, $5\% \geq P > 1\%$ ++ $1\% \geq P$

the voltage from the non treated ears would not be valid

In this series of experiments, 0 3% otic solutions were used

As is shown in Table III, the test solution for one day caused significant loss of hearing in one out of four animals Instillation for 2 days and 3 days caused severe deafness at all freuqencies (Table III)

DISCUSSION

It is difficult to extrapolate a non toxic level of topical gentamicin from the histological data such as of Wersall (1969, 1971) who used 50% gentamicin solution for 1 to 4 days and 0 3% solution for 7 to 13 days and of Webster et al (1970 1971) who used 3%, 6% and 10% solution, because cochlear impairment had already existed before vestibular disturbances became noticeable

Since their histological changes were more marked in the cochlear component than in the vestibular component it would be reasonable to assume that cochlear impairment may have occurred well below the concentration of gentamicin they used topically

Stupp et al (1973) also demonstrated severe damage of the hair cells in all turns of the cochlea following 0 1 molar gentamicin in the middle ear cavity for 3 days 0 1 molar gentamicin is equivalent to 5 4% solution With our electrophysiological technique it is much easier to detect accurately the degree of hearing loss in terms of dB scale expressed as the decrease of the cochlear microphonics

Our results from guinea pigs indicate that the toxic level of topically applied gentamicin is less than 0 3% and is effective within 24 hours

There may be some difference in suscepti bility to the drug from species to species Judging form the above results guinea pigs appear to be approximately 10 times as vul nerable as cats Similar results have been obtained following topical application of chloramphenicol on the round window of cat (Patterson & Gulick, 1963) and guinea (Gulick & Patterson 1964) It is appar however, that topically applied gentamici able to cause an ototoxic effect

Ototoxic level of the gentamicin

It is generally agreed that a normal clin dose of gentamicin in systemic use (3 mg/k body weight) does not cause any otot effect, provided the kidney is functio normally

Study of Jackson & Arcieri (1971) sho that the ototoxic level of gentamicin in hum is above 12 μg/ml of plasma

Another study by Winters et al (19 showed that the ototoxic level of gentamic serum 12 μg/ml can be reached by inject 4 mg/kg body weight

Chisholm et al (1973), injected gentam 80 mg intramuscularly into a dog of 26 kg b weight and the dog showed a peak serum le of approximately 10 μg/ml at 1 hour an peak tissue fluid level of 6 μg/ml at 3 ho following the injection His peak serum lev in good agreement with the result of Win e

No work has been published on the e ototoxic level of gentamicin in the inner fluid If we assume that the ototoxic leve the drug in the serum is the same as in inner ear fluid and just ignore all of the fact the dynamics of the inner ear fluids we calculate how much gentamicin in the b can be transferred to the inner ear through round window membrane

From our results 0 3% otic solution for day caused deafness in one out of four a mals hence we regard this as the toxic level far as the duration is concerned Since μg/ml is the level of toxic concentration in plasma (Jackson & Arcieri 1971) the inner fluids which have a volume of approximat 30 μl will need approximately 0 4 μ gentamicin to reach this toxic level and t means 0 13 μl of 0 3% gentamicin solution to be diffused to the inner ear across t membrane Only 0 4% of the total inner

has to be replaced by the otic solution in
ılla for this level to be reached

CONCLUSION

ıtical analysis of the cochlear responses
carried out and quantitative analysis of
earing loss was easily made in terms of dB
ng loss as well as of probability The
ıᵒᵉ hearing from the guinea pigs which
Ringer s in the middle ear cavity for more
one day was found to decrease signifi
y but the reason for this could not be
ined successfully The decrease was re
ble

r result indicated that even 0 3%
micin solution is toxic to the inner ear if
ed into middle ear cavity for more than 24
› There is a need to establish some
ard technique and authorized labora-
› to test the ototoxicity of all new and
dy available otic solutions

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al Scientific Meeting of the Otolaryngological Soci
Australia held in Sydney in May 1974

ZUSAMMENFASSUNG

Ototoxitat des Gentamicins tropfenweise in die
n eingefuhrt wurde an Meerschweinchen studiert
Gentamicine wurden in die eine Ringersche
ıgkeit in die zweite Mittelohrenhohle eingeführt und
Unterschied des cochlearen Mikrophonpotentials
ssen mit Hilfe von Elektroden an den Rundfenstern
le

4 ı dritten Tag wurde die Schwerhohrigkeit be
end Vom vierten Tag der Behandlung mit der Ring
hen Fluss gkeit an erfolgte eine teilweise Genesung
der Schwerhohrigkeit Als 0 3% Gentamicin in die
elohrenhohle eingefuhrt wurden erfolgten eine
signifikante Schwerhorigkeit 24 Stunden spater und eine
sehr signifikante Taubheit am zweiten und dritten Tag
Der Gebrauch von Gentamicin-Ohrentropfen in der
bisherigen Zusammensetzung sollte daher eingestellt
werden bis eine bessere Zusammensetzung gefunden
wird

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T Morı ono M D
Physiology Dept
University of Western Australia
Nedlands W A 6009

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CONGENITAL STAPES FIXATION, SYMPHALANGISM AND SYNDACTYLIA

P. Vase, S Prytz and P. S Pedersen

From the Department of Otolaryngology Odense University Hospital Odense and the Departments of Otolaryngology and Pediatrics G Rigshospitalet Copenhagen Denmark

(Received January 20 1975)

Abstract An autosomal dominant hereditary syndrome is described consisting of congenital deafness, characteristic finger and toe deformities with absence of the proximal interphalangeal joint together with syndactylia The syndrome has been observed in 5 members of a Danish family, and the study confirms the autosomal dominant trait

Symphalangism was first described by Mercier (1838) who reported a French family having only two phalanges on each finger More and more reports have been published since then on symphalangism, partly as an isolated phenomenon, partly in combination with deafness, other hand and foot deformities and malformations of the lower arm Cases have been registered in families throughout five continents, including China, Japan and Peru (Cushing, 1916, Strasburger, 1965)

The latter author has described the largest family with a total of 684 individuals, of whom 351 were affected ' A considerable number ' of these patients had congenital conductive hearing loss and operation revealed osseous fixation of the stapes

Proximal symphalangism is termed in the Anglosaxon literature "Talbot fingers ' after John Talbot who was the first Earl of Shrewsbury and who fell during the battle of Castillon in 1453 The Earl plays a part in Shakespeare's King Henry VI, but no mention is made of his finger deformity This diagnosis was confirmed at the time his grave was op[ened in] 1874

The combination of congenital stap[es fixa]tion and symphalangism was first descr[ibed by] Vesell (1960) and later by Gorlin (1970)

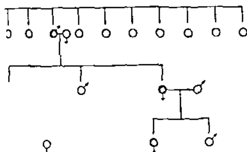
MATERIAL

The material consists of a family of 21 [...] distributed over three generations 5 [...] combination of congenital hearing impa[irment,] proximal symphalangism and syndactyl[ia. The] remaining members of the family are [...] (Fig 1)

Case I

The proband is now a 13 year-old girl w[ho has] suffered from a hearing loss since her [early] childhood and who has congenital symp[halangism] malformations of the hands and feet [The pa]tient's hearing loss brought about spe[ech re]tardation and at the age of 4 years aud[itory] treatment was commenced with good ef[fect on] speech development Audiometry s[howed] conductive hearing loss on both sides [...] discrimination loss and it was imposs[ible to] obtain a stapedius reflex on either side

At the age of 55 years the first left sid[e ex]plorative tympanotomy was performed [dur]ing which a normal ear drug was found [...] was no deformity of the bones of the [...]



1 Pedigree of the family

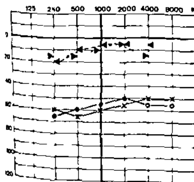
but osseous fixation of the footplate the stapes was present Indication for edectomy was not considered to be pres- at this age The hearing remained un- nged throughout the following years A her tympanotomy was carried out at the of 10 years during which it was found that rus longum incudis was elongated and the es deformed and fixed Stapedectomy ac ing to the method of House was then ormed A similar operation was done on other ear 4 months later During this pro- ure it was found that the crus longum in s was deformed and that the footplate of stapes was fixed A satisfactory permanent rovement in hearing was obtained by both rations (Fig 2)

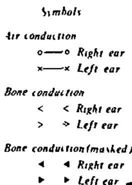
e deformities of the hands consisted of dactylia between the base of the 4 ulnar ers together with ankylosis of the middle its of the 4th and 5th fingers Corresponding symmetrical changes were present in the feet (Fig 3)

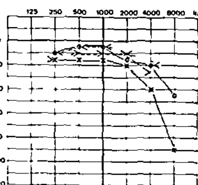
X ray examination revealed symmetrical changes with fusion of phalanx 1 and 2 of the 5th finger, which was also shorter than normal (Fig 4) The joint between the corresponding phalanx of the 4th finger was incomplete, and the remaining fingers normal apart from the syndactylia It was seen that in the wrist the capitate and hamate were fused, as were the *trapezoid and trapezium* This was so *in* both wrists, in such a way that the distal part of the wrist in actual fact merely consisted of two bones There was a corresponding fusion in the foot of the 1st and 2nd phalanx of the 3rd, 4th and 5th toes, together with fusion of the talus and calcaneum There was in addition block formation of the cuboid and lateral cuneiform

Case II

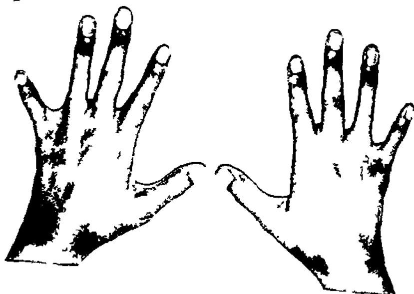
A 39 year old man, father to the proband, suffered from hearing loss from childhood Otological examination revealed a narrow external meatus, but normal ear drums The audiogram showed bilateral severe conductive hearing loss, and no reflexes could be obtained from the stapes on either side In addition the patient had syndactylia between the 4 ulnar fingers together with ankylosis of the proximal interphalangeal joints of the 4th and 5th fingers on both hands Similar changes were present *in both feet*

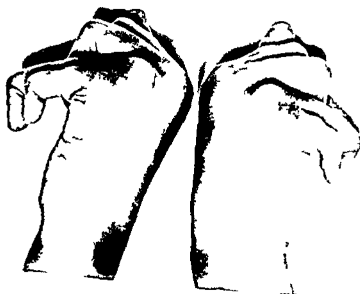


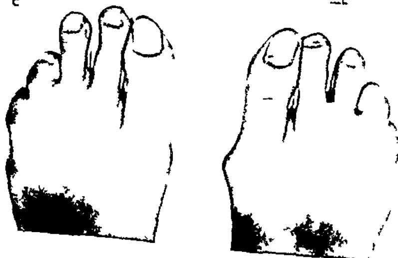




2 Pre and postoperative audiograms







F g 3 Typ cal deform and feet w h syndac yla the 4 ulnar d gits (*a c*) T lotic nterphalangeal joints and 5 h f ngers p e ent no sure of the hands (*b*)

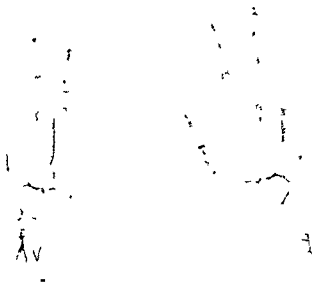


Fig 4 Roentgenogram of the hands of the patient (case I) showing fusion of phalanx 1 and 2 of the 5th finger and an incomplete joint between the corresponding phalanges of the 4th finger Note fusion of both the capitate and hemate and the trapezoid and the trapezium

III

year-old man, father to patient no II, had red from poor hearing for many years audiogram showed bilateral conductive ing loss The ear drums were fibrous but able There were symmetrical finger and leformities with syndactylia between the 4 r fingers and toes, but only the proximal of the 5th finger showed ankylosis

IV

-year old woman, sister to patient no II, had a hearing loss of approximately 20–25 ince childhood, particularly in the discant and especially in the left ear Ear drums htly fibrous but movable in Siegle's fun- No stapes reflexes The 4 ulnar fingers of h hands had syndactylia

e V

5 year old girl with congenital hearing loss, ghter of patient no IV The audiogram owed a conductive hearing loss of 40–50 dB mography showed sclerosis of the oval ndow on the left side The child had tali- s equinus-varus at birth in addition to syndactylia between the 1st and 2nd toe on both sides Syndactylia was also present between the 4 ulnar fingers, as was ankylosis of the middle joints of the 3rd, 4th and 5th fingers of both hands

DISCUSSION

Reduced hearing in childhood occurs in Europe and North America with a prevalence of 0 3–0 03 (Brown, 1973, Fisch, 1973) Approximately 50% of these cases are hereditary, the 35% autosomal recessive, 10–15% autosomal dominant and approximately 2% X-bound recessive (Fraser, 1971, Nance, 1971) The combination described of congenital stapes fixation, proximal sym phalangism and syndactylia thus belongs to the group of autosomal dominant hereditary diseases (Gorlin, 1970, Strasburger, 1965)

Histological studies of congenital stapes fixation (House, 1958, Steele, 1969) have shown a lack of development of the annular ligament and a bridge of cartilage, which becomes, at a later date, the site of ossification Congenital stapes fixation can occur as an isolated development abnormality (Bernstein, 1966), but

most often it is seen in connection with other malformations such as in these cases with symphalangism, though it can also be seen together with severe deformities such as acrocephalo-syndactylia (Bergstrom & Neblett, 1972) Congenital stapes fixation in combination with other malformations of the hands and feet, but without an accompanying symphalangism has been described by Spoendlin (1974) and Schulthess (1974) In contrast to otosclerosis, the hearing loss with congenital stapes fixation does not progress and is of a purely conductive type, the audiogram showing a typical flat curve (House, 1958).

In order to obtain more information as to the nature of congenital hearing loss it is important that any accompanying malformations be registered It is also important that in cases where bone deformities are present a possible hearing loss should be borne in mind, in order that adequate treatment can be instituted as early as possible.

ZUSAMMENFASSUNG

Es wird ein autosomales, dominantes erbliches Syndrom beschrieben, das aus angeborener Herabsetzung des Gehors, charakteristischen Deformitäten der Finger und Zehen, Mangel an proximalen Interphalangealgliedern sowie Syndaktylie besteht Das Syndrom wurde bei 5 Mitgliedern einer danischen Familie gefunden Durch Untersuchungen wird die autosomale Dominanz bestatigt

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P Vase M D
Dept of Otolaryngology
Odense University Hospital
DK-5000 Odense
Denmark

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ACUTE OTITIS MEDIA

A Clinical, Bacteriological and Serological Study of Children with Frequent Episodes of Acute Otitis Media

P Branefors Helander, T Dahlberg and O Nylen

From the Institute of Medical Microbiology and Department of Otolaryngology University of Goteborg Goteborg Sweden

(Received March 10 1975)

A series of episodes of acute otitis media was … with reference to bacterial findings and specific … responses in 48 children with histories of fre… odes before *D pneumoniae* and *H influen ae* most frequently isolated pathogens Re isola… py were often made in episodes with slow or therapeutic failure Most children harboured …ens in nasopharynx even when they had no signs of …tory tract infections Homologous relapses were …dy in few cases and never with pneumococcus type only once with *H influen ae* type b Specific responses were demonstrable generally in over 2 years of age *D pneumococcus* type 3 and *…luenzae* type b generally provoked antibody re… No levels indicating immunoglobulin deficiencies found in the children

…otitis media is a complication to an up…ory tract infection mostly confined …ildren under 10 years of age In most …ren the course is uncomplicated and the …ber of otitis episodes during their child… is low Many children, however, con… repeated infections, implying a risk of hearing impairment The reason for repeated infections is obscure in most … There have been suggestions that …nical differences in the mastoid process …n the nasopharynx or disturbances in the …l function may be of importance (for ref Bluestone & Shurin 1972)

…ome workers have pointed to the fact that …r long periods of time many children harbour in the nasopharynx the principal pathogenic bacteria found in acute otitis media and sinusitis, *Diplococcus pneumoniae*, *Haemophilus influenzae* and *Streptococcus pyogenes* (β streptococci), even though they show no signs of infection (Masters et al, 1958, Willard & Hansen 1959, Box et al, 1961, Turk & May, 1967) It has been suggested that a bacterial upper respiratory tract infection such as acute otitis media or sinusitis may depend on a viral infection which destroys the mucociliary barrier and so permits a secondary invasion by the pathogenic bacteria (Davidson 1972)

The possibility of an immature immune system with insufficient antibody formation against infecting bacteria has also been proposed by some authors as a reason for repeated middle ear infections in small children (Bjuggren & Tunevall, 1952, Sloyer et al, 1974)

In a study of the immunoglobulin levels in infancy and childhood in healthy Swedish children, Johansson & Berg (1967) showed that the IgG level remained low during the first years of life At the age of 3–5 years, the level was about two-thirds of the adult level The IgM level, on the other hand, was 70% of the adult level at one year of age, which level was usually reached at the age of four

Studies concerning the prevalence of demonstrable specific antibodies against the main pathogenic bacteria isolated in acute otitis media have shown that the amount of such antibodies is low until the children are 3–4 years old as regards β streptococci and *H influenzae* (Bjuggren & Tunevall 1952, Hansson & Holm, 1961, Branefors Helander et al , 1973) Antibodies against *D pneumoniae* have been shown to appear somewhat earlier (Gundel & Schaefer, 1931, Bjuggren & Tunevall, 1952, Sloyer et al , 1974)

The present investigation is a study of a series of acute otitis media infections in a group of children who had suffered several previous episodes of otitis media The study was performed in order

1 to reveal whether the pathogenic species isolated at the first studied acute otitis media infection could also be demonstrated at subsequent episodes An additional aim was to investigate if the children of the present study harboured in the nasopharynx the main pathogens of the upper respiratory tract (*D pneumoniae H influenzae* and β streptococci) at free intervals (no signs of infection)
2 to analyse the children s ability to react with a demonstrable immune specific response at otitis media infections by *D pneumoniae H influenzae* and β streptococci A study of the serum levels of IgG, IgA and IgM was also included
3 to investigate the possible influence of a serological response on the course of the infection and on the protection against homologous relapses

MATERIAL AND METHODS

Patients

The study comprised 48 children (27 boys and 21 girls) between 9 months and 12 years of age with clinical signs of acute otitis media The study started in March 1973 and ended in August, 1974 The longest observation period in any child was 18 months and the shortest one was 6 months All of the chil[dren] had had several episodes of acute otitis m[edia] previously most of them having had at [least] two episodes during the preceding 12 mon[ths]

In total the 48 children had 103 epis[odes] during the investigation period Unfortu[nate]ly, 20 of the recurrences occurred at [a time] when observation by us was not poss[ible] Therefore, the study comprised 83 episod[es of] acute otitis media The patients were [con]trolled after 14 days and the otitis consi[dered] healed when the tympanic membrane wa[s intact] and mobile and the child was without s[igns of] upper respiratory tract infection Audiog[rams] were taken in children with suspected he[aring] impairment

All children except one were treated [with] oral antibiotics for 10–14 days This pa[tient] had shown allergic manifestations [at] administration of different antibiotics [and] was therefore treated only with [nose] decongestants and anti histaminic d[rugs] Forty one patients were treated with [peni]cillin V 40–50 mg/kg and day in [three] equal portions Three episodes of infecti[on in] this group of children were treated with ampicillin 50–70 mg/kg and day in three e[qual] portions in one of these latter infection[s] therapy was changed from amp c'lli[n to] chloramphenicol because the pa[tient] harboured an ampicillin resistant strain [of *H*] *influenzae* type b Six patients with history [of] exanthema in connection with penic[illin] therapy were given erythromycin stea[rate] 40–60 mg/kg and day in four equal port[ions] All patients were also treated with nose [de]congestants No allergic manifestations [were] noted in any of the patients during the [in]vestigation Samples for bacterial cultu[re] were taken from the nasopharynx (NF) on [day] one in all 83 instances and on day 14 in [...] instances with the technique earlier descri[bed] (Herberts et al 1971) Samples from the [middle ear] secretion were taken on day one in 36 in[stances] tions In addition samples from the NF w[ere] taken from 38 of the 48 children at least [one] month after an acute otitis media infectio[n]

e I *Bacterial findings*

	D pn	H infl	β str	Mixed path cult	Ord gr	Neg cult	Total
ample	35	19	5	13[a]	11	–	83
ample	10	4		2[b]	3	17	36

) pn + H infl 1 D pn + β str 1 H infl + β-str 1 D pn + β str + St aur + Neiss
pn + β str + St aur + Neiss 1 H infl + β str

n the child was without any signs of re tory tract infection (these were denoted interval samples) Samples for serological yses were obtained from 47 children with ecutive serum samples taken at 59 odes of infection

erial cultivation technique

samples were incubated within 2 hours bically at 37°C on blood and gentian vio ar plates and in elevated CO_2 atmosphere ematin agar plates The cultivation and ification procedures used have been de ed previously (Branefors Helander et al) The following bacterial species were ted as potentially pathogenic *D moniae H influenzae β* streptococci *Staph aureus* In the ear samples Neis (non specified) was also denoted as po ally pathogenic The following bacteria labelled as indigenous growth Neisseria specified) *α* streptococci diphtheroids *Staph albus* and Neisseria (in the NF les)

logical tests

unoglobulin determinations All serum ples were kept at −70°C until the tests performed The IgG IgM and IgA con rations were determined by the radial im odiffusion method described by Mancini (1965) slightly modified (Nilsson 1968) preparation of the agar plates goat anti an IgG IgM and IgA sera (Hyland oratories) were utilized A standard hu serum for immunological determination plasma proteins (Behringwerke AG) was d as reference and wells containing 100 75, 50 and 25% of the appropriately diluted standard serum were included for each plate All serum samples were tested in duplicate

Pneumococcal anti-capsular antibodies were determined by the direct agglutination method The sera (0 1 ml) were serially two fold diluted on flat bottomed Linbro trays and about 3×10^7 pneumococci were added from a 6 hour old formalinized fluid culture After incubation at 37°C for 2 hours and at room temperature for another 2 hours the tests were read with the aid of a magnifying glass The initial serum dilution in the test was 1 2

H influenzae type b anti capsular anti bodies were determined by means of the indirect haemagglutination method (IHA) Untreated human ORh-erythrocytes were coated with capsular antigen b The material and the method have been described earlier (Branefors Helander 1973) The initial serum dilution in the test was 1 2

H influenzae 0 antigen antibodies were de termined by means of complement fixation (CF) test in which a mixture of bacteria from 20 non-capsulated *H influenzae* strains was used as antigen The material and the method have been described earlier (Branefors 1973 Branefors et al 1973) The initial serum dilu tion in the test was 1 15

Antibodies against β-haemolytic strepto cocci (AST) were determined by means of the anti streptolysin test (Winblad 1941)

RESULTS

Bacterial findings

The results of the bacteriological investigation are summarized in Table I The samples from

Table II *The bacterial findings at the different episodes of acute otitis media*

Pat	Age	Episode I	II	III	Free interval
1 NJ	9 m	Pn^{3}	ord gr [a]		β str
2 LE[2]	11 m	Pn^{6}	Hib	Pn^{23a}	Pn^{23} Hib
3 HH	17 m	Pn^{19}	Hi[a]	Pn	Pn^{22}
4 AE[1]	23 m	Pn^{19}			ord gr
5 AL	23 m	Pn^{6}			Pn
6 MM[3]	27 m	Pn^{19}			–
7 LK[2]	27 m				no growth
8 CA	31 m	Pn^{3a}	Pn^{23}	β str	Pn^{23}
9 MO[1]	31 m	Pn^{3}			Hi
10 AB[1]	31 m	Pn^{23}	Pn^{23a}		Pn^{23}
11 PL	31 m	Pn	Pn^{23} Hi	Pn^{19} Hi[a]	ord gr
12 FJ	3 y	Pn^{6}	Hi	Pn^{4a}	ord gr
13 HR	3 5 y	Pn^{19}	Pn^{19a}		Pn^{19}
14 BJ[1]	4 y	Pn^{3}			ord gr
15 AG	5 y	Pn^{23a}	Pn^{6}		Pn^{23}
16 DK	5 y	Pn^{29a}	Pn^{18} Hi		Pn^{18} Hi
17 OA[1]	6 y	Pn^{8}			Pn^{19} Hi
18 NL[1]	6 5 y	Pn^{3} Pn^{10}	Pn^{14} Hi[a]		Pn^{35} Hi
19 PA	7 y	Pn^{19}			–
20 PP	8 y	Pn^{3}	Pn^{7a}		Pn β str
21 TH	9 y	Pn^{3} Pn^{34}			ord gr
22 TR[3]	14 m	Pn^{19} Hi[a]	Pn^{19}	Pn β str	Pn^{19}
23 JA	28 m	Pn^{3} Hib			Hib
24 SF	29 m	Pn^{6} Hi[a]	Pn^{6} Hi		Pn^{6} Hi
25 IS	5 5 y	Pn^{5} Hi			–
26 MF	17 m	Hi	β str	Hi[a]	Pn
27 PH	22 m	Hib	Hib[a]	Pn^{23}	Hib Pn^{23}
28 MW[2]	25 m	Hi			ord gr
29 KG	3 y	Hi			Hi β str
30 ML	4 y	Hi	Pn^{14} Hi	Pn^{19a}	Pn^{9}
31 MD	4 y	Hi			β str
32 SP	4 y	Hi	Pn^{6}		–
33 LL	5 y	Hib			Hib
34 AK	5 y	Hi	Hib[a]		Pn
35 JS	7 5 y	Hib			Hib
36 AL	10 y	Hi			–
37 KJ	12 y	Hi			–
38 EF	8 5 y	Hi β str			Hi
39 MJ	4 5 y	β str			Pn^{6} Hi
40 CN	8 y	β str			ord gr
41 ST	34 m	ord gr			Pn^{6}
42 HA	4 y	ord gr	ord gr [a]	β str	Pn^{14}
43 SC	5 5 y	ord gr			
44 AJ[1]	5 5 y	ord gr			–
45 RL	6 y	ord gr			Pn^{18}
46 KB	7 y	ord gr			–
47 AW	10 y	ord gr			–
48 LJ	10 y	ord gr [a]	Pn^{3} β str St aur		β str

2 LE had one more episode with Pn and one with Hi 11 PL one with ord gr and 26 MF one with Pn
[1] [2] Signifies the number of additional episodes
[a] Free interval sample after this episode

the NF yielded *D pneumoniae* as the sole pathogen in 35 episodes (42%) and *H influenzae* in 19 (23%) while β streptococci was found as the sole pathogen in only 5 cases A culture with two or more pathogenic spe
was found in 13 infections

In 36 cases samples were taken not o
from the NF, but also from the ear secre
(Table I) Pathogenic bacteria were isola
from the ear secretion in 16 cases and in 1
these the same species were found in the N
In one child *H influenzae* and β-streptoc
ci were isolated from the ear and
β streptococci from the NF The cultiva
from the ear secretion was negative or yiel
only non pathogenic species in 20 cases w
19 of them yielded pathogens in the NF T
growth in the NF was in these cases as we
in most others recorded as moderate or hea
It was also noted that in the NF the pathog
bacteria were often found as the sole spec
All bacteriological results given in the foll
ing text are based on isolations from the N

Table II presents the bacterial findings
the first studied and the two subsequent in
tions for each of the 48 children As rega
pneumococci, the most commonly isola
serological types were 3 and *19 (9 and 1*
lates each), followed by 6 and 23 *(7 isol*
each) The seven other types found were r
resented by only one or two isolates e
(The serological typing of *Diplococ*
pneumoniae has been performed at Sta
Seruminstit Copenhagen Denmark by E
Lund, M D to whom our thanks are due
of the 30 isolations of *H influenzae* were
capsular type b, while the remainder were
capsulated Beta streptococci were often
lated in a mixed pathogen culture It is not
that *Staph aureus* was found in only
single infection In this case *(48 LJ)* S a
aureus was isolated together with β strep
cocci pneumococci and Neisseria (non spe
fied) from both the NF and the ear secret
This patient had had a tympanic memb
perforation for about a week when the samp
were taken

As may be seen from Table II the sa
bacterial species or sero-type that was isolat
at the infection first *studied was in most cas*
not observed at a subsequent *infection* F

'e III *Immunoglobulin levels in mg per 100 ml serum in 47 children with frequent acute otitis a infections*

ot s		IgG			IgA			IgM		
s)	No	Mean	S D	Range	Mean	S D	Range	Mean	S D	Range
	7	800*	374	543–1 559	49 4*	29 8	18–106	58 1	33 2	23–111
	11	781	257	469–1 250	109 5*	51 9	29–200	91 2*	34 3	59–176
	11	1 078*	361	640–1 854	106 0*	71 3	18–270	84 7	47 0	25–157
	18	1 186*	385	614–1 930	129 6*	49 7	80–278	83 5	41 0	24–168

nean values indicated with * are significantly higher ($P<0.05$) than corresponding values given for healthy swedish ten by Johansson & Berg (1967)

ance, there had often been a change from pneumococcal type to another or from umococci to *H influenzae* or vice versa n the children under 3 years of age umococci were found nearly twice as often *I influenzae* while they were about equally nmon in the older children Cultivations h ordinary growth were, with few excepts found in children over 3 years of age

After treatment, *D pneumoniae* was reated from 14 of the 47 infections where it been initially isolated In spite of its initial h frequency, type 3 was never re-isolated, was it found in a subsequent infection or in te interval in any child *H influenzae* was solated after treatment in 23 out of 30 es In five of these the strains were of sular type b In addition, *H influenzae* was lated in moderate or heavy growth after rapy in 14 cases where it had not been nd initially

At a free interval, samples from the NF re obtained from 38 children and in 29 of m pathogenic bacteria could be demonated (Table II) *D pneumoniae* was the thogen most often found (21 patients), followed by *H influenzae* (13 children) As rerds pneumococci, the same sero-types as in previous infection were found in only 4 ildren (2 LE, 10 AB, 15 AG and 24 SF) It as also observed that 3 children (8 CÅ, 16 K and 27 PN) shortly afterwards had new pisodes with the same sero-types isolated as the free interval It is noteworthy that no ss than 5 of the 6 children with *H influenzae* b infections had this micro-organism in a free interval sample taken 2–6 months later It is also notable that as many as 5 children were found to harbour β-streptococci at a free interval One of these children (48 LJ) had a new episode of acute otitis media within one month, at which infection β-streptococci could be isolated

Serological results

Immunoglobulin determinations In Table III the results of the immunoglobulin determinations are summarized It may be noted that the measured levels displayed a wide range for all age groups in both IgG, IgA and IgM However, in subsequent samples from individual children, very consistent values were obtained In none of the children in this study was general hypogammaglobulinaemia observed No IgG levels were below the normal range and only a few children had values for IgA or IgM that were lower than reported normal for uninfected children On the contrary, many individual values and also most mean values were found to be significantly higher ($p<0.05$) than those reported by Johansson & Berg (1967) for healthy Swedish children

Pneumococcal anti-capsular antibodies (agglutination test) These antibodies were determined in consecutive serum samples by means of homologous pneumococci from 22 children with isolation of this species The results summarized in Table IV show that more than half the number of children had demon

Table IV *Agglutinins against homologous serological types of D pneumoniae in consecutive serum samples from 22 children*

	Demonstrable titres	
	initially	7–14 days later
Number of samples with antibodies	13/22	17/22
Titre range	1 2–1 16	1 4–1 16

strable titres already in the initial samples Five of these children were under 3 years of age In the sample taken 7–14 days later only 4 more children showed a titre Antibodies against type 3 were found in 6 out of 7 children tested, a titre increase (>twofold) being observed in 4 of them (1 NJ, 18 NL, 23 JA and 48 LJ) In only 2 other children, infected with types 23 and 6, was a titre increase observed (10 AB and 24 SF) The brothers 13 HR and 22 TR were the only children with demonstrable initial antibodies against type 19

H influenzae type b anti-capsular antibodies (IHA-test) This test was performed in serum samples from 47 children and consecutive samples were investigated from the 6 children with isolation of *H influenzae* type b The results are summarized in Table V Eight of the 41 children (20%) with no isolation of *H influenzae* type b showed demonstrable anticapsular-b antibodies One of these children was under 3 years of age

In 2 of the 6 children with isolation of *H influenzae* type b, antibodies could be demonstrated even initially In the second sample 2 more children showed demonstrable antibodies It seems notable that 2 of the 3 children under 3 years of age showed increasing titres (2 LE and 23 JA) The third child under 3 years of age was 27 PH in whom no titre was revealed in any serum sample, despite repeated episodes and a carrier state of altogether 4–5 months duration

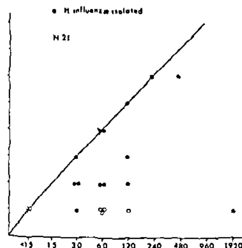
H influenzae O antigen antibodies These were determined in consecutive serum samples from the 47 children by means of the CF-test The results are summarized in Fig

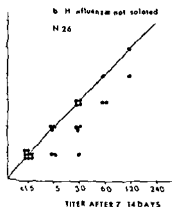
Fig 1 *a* presents the results obtained fr episodes with initial isolations of *H fluenzae* It is seen that 10 children displaye titre increase (>twofold) The children whi sera revealed a titre increase or an initial ti of over 1 30 were all over 2 and most of th over 3 years of age The 3 children without demonstrable titre were under 2 years of ag LE, 22 TR and 27 PH) The child with exceptionally large titre increase was 24 who also showed a titre increase against isolated pneumococcus type 19 In Fig 1*b* titres demonstrated in the episodes with initial isolations of *H influenzae* are p sented In 10 of these episodes *H influen* was isolated after therapy but this did seem to affect the CF-titres The mean ag the children without demonstrable initial was 43 months (9–72 months) as comp with 74 months (31–120) for those who titres This difference is statistically signifi ($p<0.05$)

Anti-streptolysin titres (AST) In Table the AS titres for the 47 children are s marized Serum samples were obtained m of the eight episodes with isolations β streptococci The results showed that t children under 2 years of age (22 TR an MF) did not get any increasing titres while other three (38 EF, 39 MJ and 40 CN) all 4 years of age, showed increasing titres (fr 1 100 to 1 400 or 1 800) In the group children from whom no β streptococci w isolated only half the number had a dem strable AST (≥50) Only 2 children both 4 years of age had a titre as high as 1 400

Table V *Agglutinin titres against caps antigen b of H influenzae tested with indi haemagglutination method in 47 children*

Isolation	Demonstrable antibodies	
	initially	7 14 days la
H infl type b	2/6 (1 8 1 32)	4/6 (1 8–1 3
No H infl type b	8/41	





f CF titres in infections with (*a*) and without (*b*) isolation of *H influenzae* Filled symbols indicate healing and open symbols therapeutic failure or slow ig

ical aspects

y six of the 83 episodes studied were con red healed at the control after 14 days rteen episodes were not completely healed r 14 days but prolonged antibiotic therapy l not considered necessary Therapeutic ure was noted in 2 children with two sodes each (22 TR and 27 PH)

The child 27 PH had two episodes at which *influenzae* type b was isolated both initially l after therapy No anticapsular b or CF- es were observed in any of the serum sam s tested The child 22 TR, who was not ated with antibiotics due to allergic manifes tations had two long lasting infections with pneumococcus 19 He had an initial titre (1 8) against pneumococcus 19 even at his first infection but no titre increase could be demon strated

In the 13 infections with slow healing, pneumococci and *H influenzae* were isolated equally often (in four of these episodes isolated together) Of the slowly healing pneumococcal otitis episodes, only one was by type 3, in a child aged only 9 months It may be noted that two of the seven slowly healing *H influenzae* infections were of capsular type b In only one of the 13 children with slowly healing episodes was a specific antibody titre demonstrable initially Six of the children, however, showed demonstrable titres in the samples taken after treatment and among those were the 2 children with *H influenzae* type b infections None of them got a new episode of otitis media with this microorganism but 2 LE had three more infections with other bacterial species isolated The children 10 AB and 24 SF had demonstrable titre increases against pneumococci but despite this immunological response, their infec tions healed slowly The same pneumococcal types were also found after therapy and both children soon got new episodes with isolation of the same sero-types Even at the free inter val samples taken 2 and 5 months later, identi cal pneumococcal sero types could be isolated from the NF

The children with episodes of acute otitis media which were considered healed after 14 days were mostly older than those with therapeutic failure or a slowly healing infection Concerning the bacterial findings in this group of children, it may be noted that all episodes with β streptococci or indigenous

Table VI *Antistreptolysin titres (units/ml) measured in 47 children*

Isolation	<50	50	100	200	400	800
β-streptococci	1	1			2	1
No β-streptococci	22	9	4	5	2	

growth were healed within 14 days, as were all but one episode with pneumococcus type 3

As regards the *serological reactions*, it may be noted that specific antibodies were generally observed in the children with pneumococcus type 3 In children with well healing *H influenzae* infections CF titres were mostly demonstrable in the initial serum sample in contrast to those with therapeutic failure or slowly healing infections

It is also notable that in most of the 17 infections which did not heal properly, at least one of the initially isolated pathogens could be re isolated after treatment, while re isolations were more infrequent among the well healing infections (12/17 as compared with 27/66) This difference is not significant (0 05<p <0 1)

Thirty-five recurrences were studied in 21 children The mean age for the children who had one more episode during the observation time was 42 months and it was 29 months for those who had more than one Seven recurrences occurred within 14 days after termination of the antibiotic treatment, and in four of them the same species were found at both infections (10 AB, 13 HR, 27 PH and 42 HA) The children 10 AB and 13 HR had episodes with isolation of pneumococci (Table II) From both children the pathogens had been isolated even after therapy at the previous infection studied Both these children had had demonstrable specific antibodies and 10 AB even a titre increase The child 27 PH had isolation of *H influenzae* type b without any demonstrable anticapsular b titres while the fourth child had an episode of acute otitis media with isolation of Neisseria as sole species Generally however bacteria of the same species were not isolated from the children at the first studied and at subsequent episodes of acute otitis media

In only a few children could an epidemiological pattern be traced The brothers 13 HR and 22 TR had altogether four episodes with isolation of *D pneumoniae* type 19 starting in the older boy and proceeding in the family for 3–4 months The children 1 NJ, 14 BJ and 4[illegible] were also siblings displaying an epidemio[illegible] cal pattern which was possible to follow about 4 months of infections with [illegible] pneumococci and β streptococci

There was also a connection between children 2 LE and 27 PH who attended same day nursery both having infections [illegible] *H influenzae* type b The girl 2 LE had infection by this microorganism 4 month[illegible] fore 27 PH was included in the study [illegible] ever, he had had three long lasting episo[illegible] with isolations of *H influenzae* [illegible] serotyped, during these months The *H* [illegible] *fluenzae* type b strain isolated from the [illegible] well as from the ear secretion of 27 PH pr[illegible] to be resistant to ampicillin Therel[illegible] this child was later treated with oral c[illegible] amphenicol with a good result How[illegible] ampicillin resistant *H influenzae* ty[illegible] was still isolated from his NF 3 months [illegible] Because of this a bacteriological examina[illegible] of the children and the staff at the nurser[illegible] made *H influenzae* type b was isolated fr[illegible] of the 10 persons examined but all t[illegible] strains including that of 2 LE turned out [illegible] normally sensitive to both penicillin [illegible] ampicillin

Eleven of the 48 children had under[illegible] adenoidectomy before the investigation [illegible] started All of them had had new epis[illegible] afterwards and in only 4 cases did the pa[illegible] report that the operation had reduced the [illegible] quency of acute middle ear infecti[illegible] Another 8 patients were operated upon du[illegible] the last 6 months of the investigation pe[illegible] In spite of this treatment all except on[illegible] them got new episodes of acute otitis med[illegible]

DISCUSSION

In the present study of children with histo[illegible] of frequent middle ear infections the result[illegible] the bacterial cultivations are in accord [illegible] other studies carried out in recent years [illegible] cerning acute otitis media with *D pneum*[illegible]

i influenzae as dominant pathogens & Rundcrantz, 1967, Howie et al, is et al, 1972)

been proposed that one of the many le reasons for frequent attacks of acute is a chronic adenoiditis (Davidson, This suggestion was based on the hat several excised adenoids from with frequent infections showed owth of the pathogenic bacteria gen isolated in this disease However, some (Box et al, 1961, Turk & May, 1967) that children without respira act infections harbour pneumococci or *enzae* nearly as often as children with fections In contrast to such results Wil- Hansen (1959), Norstedt (1967), and et al (1972) found the main patho bacteria considerably less often in children than in those with an acute In the present study was found ost of the children harboured one or of the main pathogens in their naso (NF) even during their free intervals hey showed no signs of infections This imply that children with frequent of acute otitis media are more prone children to harbour potentially enic bacteria in the NF

n the local resistance is lowered, e g by nfection these pathogens may engage s membranes and give rise to upper tract symptoms

he present study it was noted that some en who harboured pathogens during a nterval shortly afterwards got an infec which the same type of pathogen was ed This sequence was observed for in ns by various pneumococcal types ex- tor type 3 which was one of the most y found types in the present investi It should be mentioned that among the occal episodes only those caused by 3 provoked a demonstrable antibody re in most cases Carlens (1943), too, was to report antibody responses against this mococcal serotype in children over one year of age in his studies on acute otitis It may also be noted that all episodes of acute otitis with type 3 were considered healed after 14 days, except one in a child aged 9 months In none of these children was pneumococcus type 3 found after therapy The observed good therapeutic results as well as the absence of isolations at free intervals might be attributed to good immunogenic properties of this pneumococcal serotype

The therapeutical results at the seven episodes of acute otitis media with *H influenzae* type b isolations varied, with good healing in three, slow healing in two and therapeutic failure in two incidents It is interesting to note that in all but one of these patients *H influenzae* type b was found even after therapy In all children with re isolation after therapy a carrier-state was established as indicated by *H influenzae* type b isolations in samples from NF taken 2–6 months later Specific antibodies as tested by the IHA-method were demonstrable in sera of 4 patients who despite a long term carrier-state did not get a new episode with *H influenzae* type b One child in whom no serological response was observed had a second episode with *H influenzae* type b and therapeutical failure was noted at both instances In addition a carrier state for at least 3 months was seen

The explanation for most re isolations of *H influenzae* type b is most probably the comparatively low sensitivity of this bacterial species against V-penicillin The absence of recurrences despite a long term carrier state of *H influenzae* type b might be explained by the fact that in these patients a specific antibody response was present

Our observation that it is the small children who are most prone to get repeated attacks of otitis media is in agreement with the results of many other investigations (e g Bjuggren & Tunevall, 1952, Lundgren, 1972) However, homologous relapses were seen in only a few cases and these episodes mostly occurred within 30 days after the onset of the previous infection These findings are in agreement

with those reported by Kamme et al (1970) for pneumococcal recurrences

The findings in this study that demonstrable specific antibodies against the three main species (*D pneumoniae, H influenzae* and β-streptococci) were mostly observed in children over 2 years of age is well in keeping with results reported by Bjuggren & Tunevall (1952) and, as regards pneumococcal episodes of acute otitis media, also with those reported by Sloyer et al (1974) The absence of a demonstrable, specific, humoral immune response in small children with acute otitis media might be the reason why they are more susceptible to subsequent attacks of otitis media with the same sero-type or species

It has been postulated that one of the possible reasons for susceptibility to repeated middle ear infections is an immune deficiency (Davidson, 1966, Kiviranta, 1967) Kiviranta found that the average gammaglobulin levels in a group of 173 children with frequent attacks of otitis media were less than two thirds of so-called normal values On the other hand Berg et al (1971), who studied American Indian children with chronic otitis media could not find any levels in the examined children indicating immunoglobulin deficiencies Like the latter workers, we could not find any quantitative defects in the immunoglobulin levels in the children examined in the present study On the contrary, many children showed elevated levels as compared with healthy children of the same age (Johansson & Berg, 1967) These higher values may be explained as caused by the repeated episodes with acute otitis media which was the criterion for selecting the children of this study

ZUSAMMENFASSUNG

Bei 48 Kindern die eine Anamnese von haufigen Otitis media Infektionen hatten wurde eine Folge von Erkrankungen mit besonderer Berücksichtigung von bakteriellen Befunden und spezifischen serologischen Reaktionen untersucht *Diplococcus pneumoniae* und *Haemophilus influenzae* waren die am häufigsten isolierten pathogenetischen Keime Bei wiederholter Untersuchung konnten oft erneut pathogenetischen Keime isoliert werden und zwar bei Erkrankungen m… samer Heilung oder bei Therapieversagen. Die … Kinder hatten pathogene Keime im Nasopharyn… wenn sie keine Symtome either Luftwegsinfekt… wiesen Ruckfallerkrankung mit bakteriologische… fund wie bei der Ersterkrankung wurde nur in … Fallen beobachtet nie jedoch bei Infektion mit P… coccus Typ 3 und nur einmal bei *H influenzae* … Specifische serologische Reaktionen lessen sich … Hauptsache bei Kindern uber 2 Jahren … plococcus Typ 3 und *H influenzae* Typ b nefen … meisten Fallen eine immunologische Rekation her… der Untersuchung der Immunoglobuline fanden … keinen Fall Konzentrationen die auf eine man… Immunantwort andeuteten

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P Branefors Helander M D
Inst Med M crobiol
University of Goteborg
S-413 46 Goteborg
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AN EXTENDED APPROACH THROUGH THE MIDDLE CRANIAL FOSSA TO THE INTERNAL AUDITORY MEATUS AND THE CEREBELLO-PONTINE ANGLE

Z Bochenek and A Kukwa

From the ENT Department of the Medical Academy in Warsaw, Warsaw Poland

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Abstract The submeningeal approach through the middle cranial fossa to the internal auditory meatus and cerebello-pontine angle after cutting the tentorium cerebelli is described It is in some way similar to that used by Morrison & King The approach is achieved by drilling the petrous bone to the level of the compact plate of the sigmoid sinus sulcus and to the lumen of the lateral semicircular canal In this way it is possible to visualise safely the entire auditory facial fasciculus, the region of the jugular foramen, the IX, X, XI, V and VI nerves as well as the lateral surface of the brain stem In our opinion this way should be and is preferable to the translabyrinthine approach through the mastoid process

We describe an approach to the internal auditory meatus and the region of the cerebello-pontine angle beneath the meninges of the middle cranial fossa, after having drilled the upper part of the pyramid and after cutting the tentorium cerebelli

A submeningeal approach to the surface of the pyramid through the middle cranial fossa has often been used in the past, particularly for drainage of this area in the case of infection, and also in head injuries However, not until 1904 when R H Perry described the section of the VIII nerve in Meniere s disease, were various specific techniques involving this region developed, among which is the operative exposure of the internal acoustic meatus and the posterior cranial fossa by an approach through the middle cranial fossa

The most extensive advocacy and the most accurate description of these operations co from William House, who in 1961 demonstra a technique of cutting part of the VIII ne without damaging the function of the inner Describing other techniques, House speci the exact indications for determining approach to the meatus and the cerebe pontine angle in the cases of a neuroma of VIII nerve according to localization of the mour According to this classification a mour with a diameter of over 8 mm, or th extending beyond the area of the inte acoustic meatus, should be removed by an proach through the labyrinth Function of inner ear has a relative value in determi the method of approach for removing the mour mass Thus the most important meth which permits the widest approach to the f of operation must allow for the followin tors tumour size and location, continuit the facial nerve and control of bleeding in case of damage to larger blood vessels An tended approach after section of the tentor cerebelli, which was first suggested by S litz et al in 1896 is not sufficient to view region of the cerebello pontine angle adeq tely In our opinion, the approach *ad mod* House is also insufficient, especially in ca of removal of tumours in the region of bulbus of the jugular vein, as those are calized medially in relation to the aud

l fasciculus According to the description lomson & King (1973), Henderson uses oach through the pyramid when removing tumours of the posterior cranial fossa, ough details of this method are unclear approach is similar to the combined ap ich used by Morrison & King (1973) but the translabyrinthine part of the on We usually open the labyrint i the middle fossa having the possibility the approach in the direction of the id sinus

OPERATIVE TECHNIQUE

general anesthesia, a 10 cm incision is le through all the tissues in the preauricular just as in the approach through the mid cranial fossa The entire surface of the amous temporal bone is uncovered and if ambdoid suture is not readily seen in the of the mastoid angle an additional incision ade in the post auricular groove, beginning i the preceding incision and running in the on of the mastoid process Using a cut burr a plate of bone approximately 5 5×3 is next removed

After exposing the meninges a 20% solution Mannitol is given i v approximately 1 0/kg ly weight A fall in intra cranial pressure is after giving Mannitol and this allows separate of the meninges from the bone ce and to raise the temporal lobe in order expose the petrous part of the temporal ne The dura mater is separated from the terior surface of the pyramid to the level of sulcus of the superior petrosal sinus from ove and the hiatus of the facial canal from front The procedure here is for decom ession of the VII nerve according to Pulec) or in exposing the internal auditory s for section of part of the VIII nerve cording to Fisch (1970) Next the superior osal sinus is gently dissected away from its lcus beginning from its orifice (to the gmoidal sinus) and progressing in the direc on of the pyramidal apex

Drilling the petrous bone is be

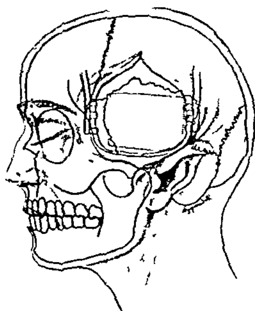


Fig 1

opening the mastoid antrum to the level of th compact plate of the sigmoidal sinus sulcu unveiling the lumen of the lateral semi-circula canal Its course is a very important landmar in the successive steps of the operation Afte opening the vestibule, the fundus may be reac ily located and the internal auditory meatu which is not opened until a sufficient amount o bone has been removed for good visualizatio of the tumour The removal of bone is begi laterally in relation to the internal audito canal and progresses towards it Such mea ures ensure greater safety with regard to t VII nerve due to the fact that even in openi the internal auditory canal the vestibular pa of the VIII nerve are located in the extren lateral side in the fasciculus

After a sufficient amount of bone has be removed the dura mater (tentorium cerebel is gently incised parallel to the upper border the pyramid Next both flaps of meninges a separated and supported by suture thread

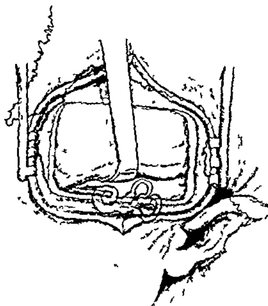


Fig 2

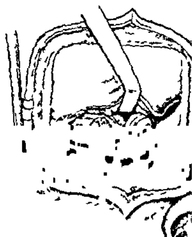
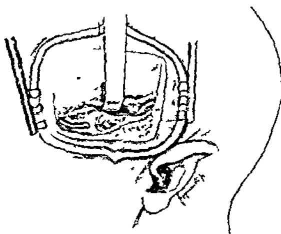


Fig 4

Such an approach gives us an exact view of the auditory-facial fasciculus, located in the field of operation Inferiorly and somewhat laterally, the sigmoid sinus is seen together with the IX, X and XI nerves, while supero medially and from the top the V, VI and sometimes the III nerves Somewhat deeper in relation to these structures is the cerebellum and the posterior inferior cerebellar artery With further removal of pyramidal bone in the direction of its apex it is possible to visualize the basilar artery an brain stem



g 3

When the access to the tumour is suffici large and all the anatomical structures in internal auditory canal and the area of the a have been identified, we may then procee remove the tumour The decision whethe remove the mass in fragments or in its entir depends on its size and vascularity Quite of it is possible to remove the tumour toget with its capsule from the surrounding tiss fairly readily Blood vessels entering t capsule or those already damaged earlier a coagulated by the use of a bipolar electrod The separation of a large tumour is usuall impossible, so that fragmentary removal i necessary, and in this way the access to th tumour is improved After removal of th tumour mass and complete haemostasis it n necessary to check the integrity of all th nerves, particularly the facial nerve The nex step is to suture the edges of the incised meninges (tentorium cerebelli) Temporal fascia is placed on the opened surface of the a space of the middle ear and petrous bone By this means additional separation of the middle ear from the cranial cavity is achieved which in turn guards against the liquorrhea and the pos sible spread of infection from the middle ear to the meninges A delay of 15–20 min is neces-

, to allow for the dilatation of the temporal The removed plate of temporal bone is aced in its former place and is sutured with A rubber drain is placed into the ' space for about 24 hours Soft tissues sutured in layers In the postoperative l (2–3 days) Mannitol is given i v 1 g/kg ' weight All patients receive antibiotics lactically

DISCUSSION

ne removal of tumours of the VIII nerve nding beyond the internal acoustic meatus for tumours of the cerebello-pontine angle performed through a different approach from which have been previously described is modification creates a new and improved ssibility for surgical intervention in the reg of the cerebello pontine angle Thus the of the upper part of the pyramid up to level of the bony plate of the sigmoid sinus terally and to the lumen of the horizontal micircular canal medially, enables the vis lization of the entire auditory facial sciculus the bulbus of the jugular vein the X, XI V VI and III nerves In this way it is to visualize the anterior surface of the ulum and the lateral recess of the fourth ricle as well as the lateral surface of the ain stem The anterior inferior cerebellar tery is also well seen along its entire length om the basilar artery to its loop in the area of he orifice of the internal acoustic meatus

The approach described was used exceptionally only in the situation when, according to use (1964) the approach through the middle fossa was found to be insufficient during course of the operation This was the case when the tumour was found to be larger than anticipated and extended beyond the internal acoustic meatus It is considered that this should always be the routine approach when the tumour is over 8 mm of diameter and demands a wider approach This approach to the internal acoustic meatus and the anterior part of the posterior cranial fossa is considered to be better than that passing through the mastoid process and the labyrinth, for the following reasons

1 it gives a better orientation in the field of the operation,
2 it can be extended at any given moment,
3 it creates wider visualization of the area of the cerebello pontine angle, which allows freer manoeuvering to achieve haemostasis and especially in haemorrhage from the posterior inferior cerebellar artery or its branches,
4 removal of part of the temporal bone can be used as the first step (decompression) in the case of large tumours in the posterior cranial fossa,
5 it creates the possibility of visualization of all anatomical structures in this region from a better angle,
6 it enables a wider and thus a better approach to the internal acoustic meatus and the auditory facial fasciculus along its entire length, which is important in preserving the continuity of the VII nerve,
7 it creates facilities for decompression of the VII nerve,
8 communication with the middle ear is the same as in the approach through the labyrinth but with this technique it is easier to separate these two spaces, namely the cranial fossa and the middle ear, by placing temporalis fascia or a frag ment of fascia lata on the surface of the pyramid,
9 it is well tolerated by patients,
10 in the case of complications it is easy to achieve access to the operated region

RÉSUMÉ

Les auteurs decrivent la voie submeningeale de la fosse cerebrale moyenne pour aborder le conduit auditif interne et l angle ponto-cerebelleux après l incision de la tente du cervelet La voie ressemble un peu à celle utilisee par Morrison et King On procède à fraiser le rocher jusqu au niveau de la lame compacte de la gouttière sigmoide et jusqu à la lumière du canal semi-circulaire externe On obtient ainsi en toute securité – une vue

suffisamment large sur le fascicule acoustico facial, la region du trou dechiré posterieur, les nerfs IX, X, XI, V, VI et la partie laterale du tronc cerebral Les auteurs trouvent cette voie préférable à la voie trans labyrintique à travers la mastoïde

ZUSAMMENFASSUNG

Der submeningeale Zugang durch die mittlere Schädel grube zum inneren Gehorgang und zum Kleinhirn bruckenwinkel nach der Inzision des Tentorium cerebelli wird beschrieben Dieser Zugang entspricht in gewisser Weise dem, der von Morrison und King benutzt wird Der Zugang wird durch das Bohren des Felsenbeins bis zum Niveau der Lamina compacta des Sulcus sinus sigmoidei und zum Lumen des Canalis semicircularis lateralis erreicht Auf diese Weise ist es ohne Gefahr moglich, den ganzen Fasciculus acustico-facialis, die Gegend des Foramen jugulare, den N IX, X XI, V und VI sowie auch die laterale Flache des Hirnstamms sicht bar zu machen Unserer Meinung nach ubertrifft der beschriebene Zugang den translabyrinthären Zugang durch den Mastoid

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Z Bochenek M D
Dept of Otolaryngology
Medical Academy of Warsaw
Filtrowa 44/2
Warsaw
Poland

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FIRING MECHANISMS IN THE SINGLE VESTIBULAR NEURONS IN THE CAT

I Matsuoka[1] E F Domino[2] and M Morimoto[1]

From the [1]Department of Otorhinolaryngology Faculty of Medicine Kyoto University Kyoto Japan and Michigan Neuropsychopharmacology Research Program [2]Department of Pharmacology School of Medicine University of Michigan Ann Arbor Mich USA

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bstract Spontaneous unitary discharges in nucleus vestibularis lateralis (NVL) neurons were studied in locally anesthetized cats The mean±S E firing rate of spontaneous unitary discharges of NVL neurons was 19 8±0 9 Hz bout 12% of NVL neurons showed a random firing The spontaneous unitary discharge rate of the *gamma* distribution at lambda−2 was relatively low However the others were high Patterns of peak interval time of the distribution with one peak and the interpeak interval time of those with two and three peaks were almost the same

he physiological reaction of the vestibular nerve and its nuclei to caloric stimulation and rotatory stimulation has been well documented Adrian 1943 Fredrickson et al 1966 Lowenstein & Sand 1940*a b* Matsuoka 1967 1969 1971 Shimazu & Precht 1965 Weber & Steiner 1965) However there is still paucity of data regarding the firing mechanisms of single neurons in the vestibular system Most investigators who examined the evoked activity of single neurons have commented on the background firing rate though usually briefly (Adrian 1943 Lowenstein & Sand 1940*a b* Fredrickson et al 1966 Matsuoka 1967 1969 1971 1972*a b c* Matsuoka et al 1971 1972 1973 Shimazu & Precht 1965 Weber & Steiner 1965)

Gamma distribution at λ=2
The distribution pattern Γ is the theoretical gamma distribution pattern at λ=2 and formula diagrammed in Discussion

Spontaneous activity is a striking feature of the sensory portion of the nervous system Long after specific stimuli have been discontinued, neurons in various afferent areas continue to discharge Appropriate terminology for this firing has not been formulated The term spontaneous is widely used and the difficulties with its use are generally appreciated Yet it appears preferable to other terms such as background maintained or on going

Lowenstein (1940) and Adrian (1943) reported that the frequency of spontaneous unitary discharges in the vestibular nerve and vestibular nuclei is very high even in barbiturate anesthetized animals However, Shimazu & Precht (1965) and Matsuoka (1967 and 1969 reported the frequency of spontaneous unitary discharges to be relatively low and some of those neurons are completely silent In the present experiments spontaneous unitary discharges were recorded in the nucleus vestibularis lateralis (NVL) of locally anesthetized and decamethonium immobilized cats Spontaneous unitary discharges were described in terms of their firing rate

METHODS

Twenty adult cats of both sexes, weighing 2 to 4 0 kg were used All surgery was performed

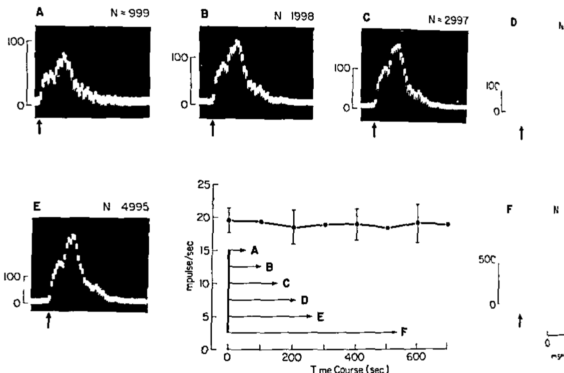


Fig 1 Reproducibility of the time histogram of a lateral vestibular neuron Total number of spikes in each distribution varied between 999 and 9990 Note that the mean firing rate and the shape of the histogram from A to F a[re the] same Calibration as noted

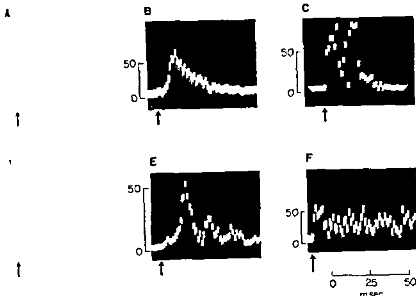
under diethylether–oxygen anesthesia The trachea and the right femoral artery and vein were cannulated The mucous membrane of the middle ear cavity of the right side was removed through an approach via the right bulla A small concentric bipolar electrode was inserted into the lateral ampullar nerve with the aid of a microscope

The recording microelectrode was gold-tipped stainless steel wire with an electrical resistence between 0 75 and 10 MΩ The recording electrode was inserted into the right vestibular nuclei, primarily NVL, with the aid of a stereotaxic instrument, using the stereotaxic coordinates of a brain atlas (Snider & Niemer, 1961)

The recording microelectrode was connected through a cathode follower to a Grass P-5 amplifier and the potentials displayed on a Tektronix dual beam oscilloscope An amplitude discriminator was used to convert amplitude and duration (TMC Model 605, 606) The output was fed into a CAT 400B computer and date printer (TMC, Model 500A) w[hich] yielded an on line interspike interval [time] histogram of single unit activity All data [dis]played on the oscilloscope were photograp[hed] on Kodak plus X film using a Grass re[cording] camera The data from the CAT 400B comp[uter] were also displayed on a dual beam os[cil]loscope and recorded on Polaroid film

One sec or 625 msec were adopted as sweeping times of 400 data points on the in[ter]val time histogram Each point contained [2.5] msec or 2 5 msec of interval time After operative procedures were completed 0.[5%] lidocaine was applied at all wounded ed[ges] after which the animal was immobilized w[ith] decamethonium (1 0 mg/kg/hr i v) and art[ifi]cially ventilated Body temperature w[as] maintained at 36° to 38°C by means of an a[u]tomatic heating pad (German Rupp Indust[ries] Inc , Model K 1 3)

At the end of each experiment 22 5 V d[c] was applied to the recording electrode so th[at] the site of recording could be confirm[ed]



2 Types of interspike interval time distributions of rent lateral vestibular neurons (total spikes in each ibution=999) A *gamma* distribution at λ 2 B monomodal distribution C bimodal distribution D exponential distribution E trimodal distribution and F rectangular distribution Calibration as noted

ologically A detailed description of the ex-mental procedures can be found in a previ paper (1973)

RESULTS

ptaneous unitary discharges from NVL lly anesthetized animals were mainly bi sic but monophasic spikes were observed asionally The majority of the neurons e 0 2 to 1 5 mV in amplitude ranging from to 70 0 Hz The mean frequency±S E of neurons was 19 8±0 9 Hz Silent neurons the low frequency neurons below 0 1 Hz e not studied

t is difficult to satisfy the mathematical re rements for a stationary process in view of relatively limited size of sampling of eous activity It was necessary that was a uniform development of the inter stogram over the period of analysis The of the histogram remained constant As in Fig 1 the mean rate of the discharge the period analysed was stable The his-ram of intervals accumulated uniformly, although the total number of spikes in each distribution was different As there was no difference in area size between 999 to 9990, the former was chosen for convenience The interval time histogram analysed at channel widths of 1 56 msec or 2 50 msec was classified into the following six types, depending upon the in shape group A showing *gamma* distribution at λ=2 and histogram of interspike interval of the NVL neuron fits the theoretical gamma distribution at λ=2 (Fig 2A), group B with a monomodal distribution has a peak in a histogram and the peak interval time of this histogram is 12 5 msec (Fig 2B) group C with a bimodal distribution has two peaks and the peak interval times of this histogram were 10 0 msec and 22 5 msec (Fig 2C), group D with an exponential distribution fits the theoretical ex ponential distribution (Fig 2D), group E with a trimodal distribution has peaks and the peak interval times of this neuron were 15 0 msec, 32 5 msec and 45 0 msec (Fig 2E) group F with a rectangular distribution has no peak, which means that the firing interval is not regular, but random (Fig 2F)

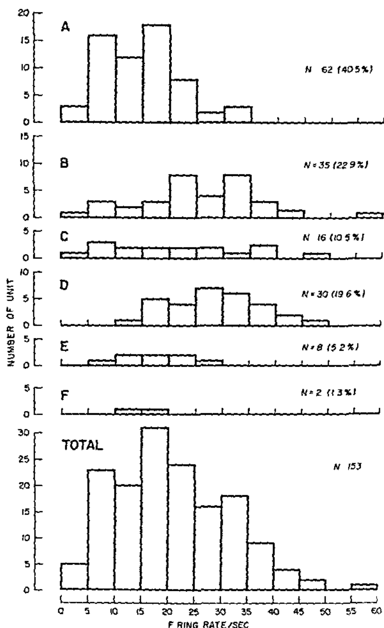


Fig 3 Relat onsh p between the [cut off] time histogram and the distr[cut off] spontaneous unitary d scharges of [cut off] lar neurons Each histogram ha[cut off] quency distribution of the mean [cut off] discharges comprising famil es on [cut off] The histogram for the entire [cut off] charges obtained by pool ng the [cut off] shown at the bottom

About 40% of the neurons exhibited the pattern of *gamma* distribution at $\lambda=2$ Of 153 neurons recorded from the NVL, the number of neurons showing *gamma* distribution at $\lambda=2$, distribution with monomodal bimodal, exponential distribution and distribution with trimodal, was 62 (40 5%) 35 (22 9%) 16 (10 5%), 30 (19 6%), and 8 (5 2%), respectively The two remaining neurons (1 3%) showed a rectangular distribution pattern (Fig 3) Sixty-two out of 153 neurons showed a *gamma* distribution at $\lambda=2$ The mean firing rate of this group was relatively low ranging from 35 0 Hz and 13 0±0 8 Hz (S E) That [cut off] out of 153 neurons had a monomodal d[cut off] tion The mean firing rate±S E of this [cut off] was 25 2±1 5 Hz and ranged from 0 1 t[cut off] Hz The peak interval time of this grou[cut off] 10 0, 12 5 15 0 20 0 and 30 0 msec (Fig [cut off]

Sixteen out of 153 neurons revealed [cut off] modal distribution The mean firing rate [cut off] of this group was 21 3±2 7 Hz The peak [cut off] val time is shown in Fig 4B The first p[cut off] this group had a very short interval [cut off]

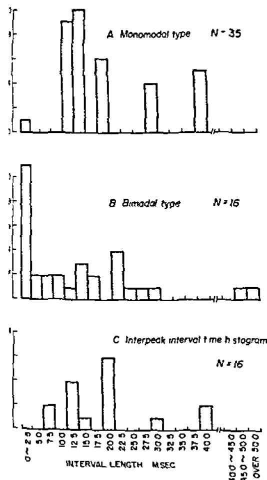


Fig 4 Predominant interspike time histogram of various ...of vestibular lateralis neurons Note that the length of interpeak time interval with monomodal distribution ...the same (A and C) as with bimodal distribution

...sec The interpeak interval time of each ...ak is shown in Fig 4C This pattern was ...most the same as the peak interval time ...stogram observed in the monomodal (Fig ...A)

Thirty out of 153 neurons showed an exponential distribution The mean firing rate±S E of this group was 28 5±1 6 Hz Eight out of 153 neurons had a trimodal distribution The mean firing rate±S E was 17 5±1 6 Hz The peak and interpeak interval times are shown in Table I The pattern of this interpeak interval time was the same as observed in monomodal distribution Only two out of 153 neurons showed a rectangular distribution The unitary discharge rate of this group was relatively low and the discharges were fired at random

DISCUSSION

The mean firing rate±S E of spontaneous unitary discharges in NVL of locally anesthetized cats was 20 0±0 9 Hz This figure is almost the same as that described by Matsuoka (1967, 1969), who reported values of 19 3±2 5 Hz (N=62) and 21 4±0 6 Hz (N=299) A significant decrease in the frequency of the spontaneous unitary discharges of NVL neurons induced by administration of barbiturates has also bee confirmed by Matsuoka (1967)

It is well known that neurons with an exponential distribution pattern fire at random and that the activity of neurons with a *gamma* distribution at λ=2 can be explained by the feedback transmission process within a closed circuit, Hagiwara (1950) Naito (1966), Oomura et al (1964 and 1967) Takaori et al (1968) and Sasa (1969) It is also well known that in the vestibular system the vestibular hair cells are excitatory to NVL nwurons and the

Table I *Peak and interpeak interval time in trimodal neurons (N=8)*

Interval length (msec)	Peak interval time	Interpeak interval time
< 2 5	4	0
– 5 0	0	0
– 7 5	0	0
10 0	1	0
12 5	0	9
15 0	5	0
17 5	0	5
–20 0	0	0
–22 5	2	0
–25 0	0	0
27 5	0	0
30 0	1	1
32 5	5	0
35 0	0	0
37 0	1	0
–40 0	0	0
>40 0	5	1
	24	16

NVL neurons are inhibitory (directly or indirectly to the vestibular hair cells) The recurrent collateral and cerebellum system to NVL neurons also has the same feedback system In the present experiments, about 20% of the NVL neurons showed a pattern of exponential distribution which indicates that only 20% of the NVL neurons fired at random in the animal at rest, while the remaining neurons are associated with feedback mechanisms About 40% of the neurons with the *gamma* distribution at $\lambda=2$ were observed The same distribution was observed in the nucleus tractus spinalis and nucleus sensorius superior (Takaori et al, 1968, Sasa, 1969) As shown in Fig 3, the interval distribution of each of the input pulse trains is assumed to be a *gamma* distribution at $\lambda=2$

$$P(T)=\frac{K^{a}T^{a-1}e^{-KT}}{T(a)}\ (0\leq T<\infty)$$

where T=length of interval, $p(T)$=probability of interval length T, and K, a are the adjustable constants The important parameter determining the shape of the distribution is the constant a, the value $a=2$ is chosen corresponding to the value obtained experimentally for peripheral cell discharges (Kuffler et al, 1957, Bishop et al, 1964) For simplicity, K is set equal to unity This *gamma* distribution at $\lambda=2$ is the shape of the assumed interval distribution for the impulse train of each excitory input fiber (Bishop et al, 1964) In the present experiments, the proportion of these neurons was much greater than in the data from Fujita et al (1968), who reported that the neurons in the NVL showed only a monomodal distribution The peak interval times of the distribution were 8 4 msec, 24 6 msec, 26 3 msec, 41 9 msec, and 54 5 msec, respectively

The peak interval time observed with the monomodal distribution was relatively longer than that in the data from Fujita et al (1968) However, the pattern of this group was almost the same The peak interval of this group had almost the same value of the interpeak interval time as was observed with the bimodal and trimodal distribution The excitatory feedba system in these neurons presumably accou for these phenomena

ZUSAMMENFASSUNG

Die Spontanaktivität von Neuronen im Nucleus Ve bularis Lateralis (NVL) von lokal narkotisierten Ka wurde untersucht Die durchschnittliche Entladungsra s f der Spontanaktivitat von diesen Neuronen war 1 0 9 Hz Die Spontanaktivitatsraten der Neuronen *Gamma* Verteilung ($\lambda=2$) zeigten waren re niedrig die der Neuronen aber hoch Die Formen Spitzenintervalezeit (Kurven mit einer zwei und Spitzen) waren fast gleich

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I Matsuoka, M D
Dept of Otolaryngology
Faculty of Medicine
Kyoto University
Kyoto 606
Japan

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EFFECTS OF CHOLINERGIC AGONISTS AND ANTAGONISTS ON NUCLEUS VESTIBULARIS LATERALIS UNIT DISCHARGE TO VESTIBULAR NERVE STIMULATION IN THE CAT

I Matsuoka,[1] E F Domino[2] and M Morimoto[1]

From the [1]Department of Otorhinolaryngology Faculty of Medicine Kyoto University Kyoto Japan and Michigan Neuropsychopharmacology Research Program [2]Department of Pharmacology School of Medicine University of Michigan Ann Arbor Mich USA

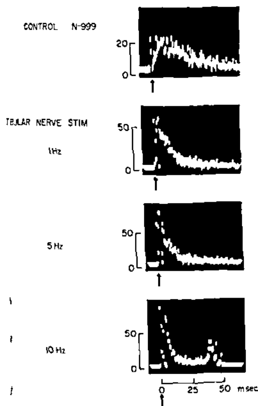
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Abstract Unit discharge in nucleus vestibularis lateralis (NVL) was studied in locally anesthetized cats The mean firing rate±S E of spontaneous unitary discharges of NVL neurons was 19 8±0 9 Hz and that of spinal transected animals was 18 0±1 6 Hz The mean firing rate of these neurons in animals with cerebellum ablation and the administration of scopolamine in a dose of 0 5 mg/kg i v was relatively low After administration of physostigmine in a dose of 25 μg/kg i v the firing rate was enhanced The effects of physostigmine were antagonized by scopolamine About 95% of NVL neurons activated by single electrical shocks to the ipsilateral vestibular nerve showed regular firing following administration of physostigmine These neurons changed to a *gamma* distribution at $\lambda=2$ following administration of scopolamine

It has been demonstrated that neurons in the vestibular nuclei are excited by the local administration of acetylcholine and/or cholinesterase inhibitors such as physostigmine given parenterally (Weber & Steiner, 1965 Yamamoto 1967, Matsuoka et al , 1973) The histochemical localization of acetylcholinesterase (AChE) in the nucleus vestibularis appears complex AChE levels are high in the lateral and superior vestibular nuclei, but low in the medial and inferior nuclei (Friede, 1966) In nucleus vestibularis lateralis (NVL) almost all of the AChE are contained in the cell bodies of Deiters giant neurons Shute & Lewis (1960) and Ross (1969*a* *b*) have also reported that AChE is present in the vestibular ganglion of Scarpa Matsuoka et al (1972 19[illegible]) have also reported that the content [illegible] cholinesterase, AChE, choline acetyltra[illegible] ferase and total cholinesterase was higher in [illegible] NVL than in the vestibular nerve including [illegible] vestibular ganglion of Scarpa Steiner & We[illegible] (1965), Yamamoto (1967) and Matsuoka et [illegible] (1973) have reported that NVL neurons a[illegible] vated by vestibular nerve stimulation w[illegible] also stimulated by the administration [illegible] acetylcholine The present report descr[illegible] studies on the firing mechanisms of the N[illegible] neurons following vestibular nerve stimula[illegible] and the intravenous (i v) administration [illegible] cholinergic agonists and antagonists

METHODS

Twenty five cats of both sexes weighing 2[illegible] 4 0 kg were previously confirmed to be n[illegible] regarding voluntary movement The opera[illegible] procedures and recording techniques w[illegible] similar to those described previously u[illegible] lidocaine locally anesthetized and dec[illegible] ethonium immobilized cats For vestib[illegible] nerve stimulation a small concentric bip[illegible] electrode was inserted into the lateral amp[illegible] nerve with the aid of a microscope after w[illegible] it was fixed with dental cement



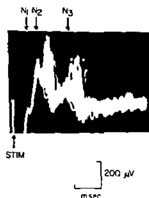


Fig. 1. Changes in interspike interval distribution following vestibular stimulation. A total of 999 discharges were recorded from the same single neurons. A: ipsilateral vestibular nerve was stimulated with single shocks 5 and 10 of 0.05 msec duration and 3.5 V. Evoked potential and evoked unitary discharges are illustrated. Twenty sweeps were superimposed. Note that the evoked unitary discharges were elicited on the monosynaptic potential (N_2) and polysynaptic potential (N_3). Time base and voltage calibration are as shown in the figure.

The administration of physostigmine in a dose of 25 μg/kg and scopolamine in a dose of 0.5 mg/kg was given i.v. A square pulse of 0.05–0.1 msec duration at 1, 5, and 10 Hz was applied to the ipsilateral vestibular nerve. The statistical validity of the distribution pattern of the histogram was determined using a Chi square test. Changes in unit firing rate caused by electrical stimulation of the vestibular nerve were determined following the formula described below and were confirmed according to the Chi square test using a criterion of $p<0.05$.

If the firing rate is below ten, $\chi^2=\frac{(|F_0-F_e|-1)^2}{F_0+F_e}$

If the firing rate is over ten, $\chi^2=\frac{(F_e-F_0)^2}{F_0+F_e}$

F_0=total number of the spontaneous unitary discharges for 5 sec before stimulation, F_e=total number of the unitary discharges for 5 sec during and after stimulation. In some animals, spinal section and ablation of the cerebellum were performed under diethyl ether–oxygen anesthesia.

RESULTS

Spontaneous unitary discharges from NVL in locally anesthetized, decamethonium immobilized artificially ventilated animals were observed. These data have been reported previously (Matsuoka et al., 1973). One hundred and fifty three neurons in NVL were classified according to the distribution pattern. The interval distribution of unitary discharges in NVL consisted of the following six groups: *gamma* distribution at λ=2, monomodal, bimodal, trimodal, exponential, and rectangular distributions. Thirty-eight of the neurons tested were responsive to the vestibular nerve stimulation with a frequency of 1, 5 and 10 Hz. The neurons responsive to vestibular nerve stimulation were classified into two responses: facilitatory and inhibitory types. The facilitatory types could be subdivided into three patterns: type I, in which the firing rate increased

Table IV *Distribution pattern of spike intervals for the same neurons before and after administration of physostigmine (25 µg/kg i v) and scopolamine (0 5 mg/kg i v)*

Control	After admin of physostigmine	After admin of scopolamine	No of neurons
Gamma distribution	Distribution with monomodal	*Gamma* distribution	11
Distribution with monomodal	Distribution with monomodal	*Gamma* distribution	4
Distribution with bimodal	Distribution with monomodal	*Gamma* distribution	2
Exponential distribution	Distribution with monomodal	*Gamma* distribution	2
Total			19

same neurons before and after administration of physostigmine and scopolamine is summarized in Table IV The *gamma* distribution at $\lambda=2$ and the bimodal and exponential distribution changed to the monomodal distribution pattern after administration of physostigmine These neurons also changed to the *gamma* distribution pattern at $\lambda=2$ following scopolamine

Spinal cord transect at the C_1 to C_2 level did not change the spike interval distribution The mean firing rate ± S E of this group was 18 0 ± 1 6 Hz, which is almost the same as the control (Table V) The pattern of spike interval after ablation of the cerebellum is summarize[d] in Table VI The percentage of neurons show[ing] a *gamma* distribution at $\lambda=2$ fell to 2 9% while neurons showing monomodal bimod[al] and exponential distributions increased [to] 38 6%, 31 4% and 27 1%, respectively

DISCUSSION

All NVL neurons analysed were responsive [to] electrical stimulation of the ipsilateral vesti[bu]lar nerve at 1, 5, and 10 Hz The facilitato[ry] type dominated These results are in agreeme[nt] with those described by Adrian (1943) Lowenstein & Sand (1940*a b*) Shimazu [&] Precht (1965) and Matsuoka (1967 and 1969) The spontaneous unitary discharges of NV[L] neurons showed a marked increase in the fir[ing] rate after i v administration of physostigmin[e] On the other hand, scopolamine reduced NV[L] neuronal firing considerably These data para[l]lel the data of Weber & Steiner (1965) Yamamoto (1967) and Matsuoka et al (197[ ]) The difference between physostigmine a[nd] scopolamine was confirmed by observing th[e] time course of the responses of the sa[me] neurons by giving the drugs in sequence

All 19 neurons classified into three facil[ita]tory types change their firing into monom[o]dal distribution after administration [of] physostigmine They then changed into

Table V *Distribution pattern of spike intervals after spinal section (C_1–C_2) in the nucle[us] vestibularis lateralis*

Discharge rate (Hz)	*Gamma* distribution	Distribution with Monomodal	Distribution with Bimodal	Exponential distribution	Number of neurons *n* (%)
0– 5	4	0	0	0	4 (6 2)
5–10	7	0	2	0	9 (13 8)
10–15	9	1	1	1	12 (18 5)
15–20	6	2	2	1	11 (16 9)
20–25	2	5	4	3	14 (21 5)
25–30	5	2	1	2	10 (15 4)
30–35	1	0	0	1	2 (3 1)
35–40	0	0	0	0	0
40<	1	2	0	0	3 (4 6)
Total	35 (33 8%)	12 (18 5%)	10 (15 4%)	8 (12 3%)	65

Mean firing rate ± S E – 18 0 ± 1 6 Hz (*N*=65)

Table VI *Distribution of spike intervals after ablation of the cerebellum*

Discharge rate	Gamma distribution	Distribution with		Exponential distribution	Number of neurons n (%)
		Monomodal	Bimodal		
5	0	5	0	8	13 (18 6)
10	2	13	15	9	39 (55 6)
15	0	5	7	2	14 (20 0)
20	0	2	0	0	2 (2 9)
25	0	2	0	0	2 (2 9)
30	0	0	0	0	0
35	0	0	0	0	0
Total	2 (2 9%)	27 (38 6%)	22 (31 4%)	19 (27 1%)	70

Mean firing rate ±S E 8 1±1 9 Hz (N=70)

gamma distribution at λ=2 following scopolamine The dominant distribution pattern of the spike interval after administration of physostigmine was the monomodal, while the distribution of spike interval after administration of scopolamine was the *gamma* distribution at λ=2 The distribution pattern of spike intervals after spinal section was almost the same as in intact animals On the other hand, the dominant pattern after ablation of the cerebellum was of the exponential type These results support the assumption that neurons in NVL behave similarly to those in which the vestibular nerve is stimulated following the administration of physostigmine

It is well known that neurons with an exponential distribution pattern fire at random The activity of neurons with a *gamma* distribution at λ=2 is best explained by a feedback transmission process within a closed circuit (Matsuoka et al 1975) Therefore 30% of the NVL neurons fired at random following ablation of the cerebellum The relatively large number of neurons in the NVL appears to be associated with such feedback mechanisms both in the control and the spinal transected groups

Apparently spontaneous unitary discharges recorded from NVL fire with some feedback system In the present experiments, NVL neurons responded most effectively to electrical stimulation of the vestibular nerve at frequencies of 1 5 and 10 Hz

The time interval histogram of spontaneous unitary discharges in about four-fifths of NVL neurons showed a *gamma* distribution at λ=2 pattern The monomodal, bimodal and trimodal distribution and the majority of the spike intervals in these types of neurons were in the order of 25 msec and 50 msec The peak interval time of the monomodal distribution and the interpeak interval time of the bimodal and trimodal distribution was in the order of 2 5 msec, 10 msec, 20 msec, 30 msec, and 40 msec respectively There are also several types of closed circuits in these feedback systems and a short interval less than 2 5 msec revealed a closed circuit in the same NVL The role of the other reflex influences of physostigmine and scopolamine given parenterally needs to be studied further Nevertheless, the present study indicates an important cholinergic mechanism in relation to their portion of the vestibular system

ZUSAMMENFASSUNG

Das Entladungsmuster von Neuronen im Nucleus Vestibularis lateralis (NVL) wurde an Katzen in Lokalanästhesie untersucht Die mittlere Spontanaktivität dieser Neurone betrug 19 8±0 9 spikes/sec beim normalen Tier und 18 0±1 6 spikes/sec bei Tieren mit spinaler Durchtrennung Die mittlere Entladungsrate dieser Neurone war relativ niedrig bei Tieren mit abgetragenem Cerebellum und auch nach Verabreichung von 0 5 mg/kg Scopolamin (i v) Nach Eingeben von 25 μg/kg Physostigmin (i v) fand eine Erhöhung der mittleren Entladungsrate statt Scopolamin hob den Effekt von Physostigmin auf Ca 95% der NVL

Neurone die durch elektrische Einzelreize des ipsi lateralen N Vestibularis aktiviert wurden zeigten nor *male Entladungsraten nach Eingeben von Physostigmin* Diese Neurone wurden durch Scopolamin zu einer Gamma Verteilung (λ−2) verandert

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I Matsuoka M D
Dept of Otolaryngology
Faculty of Medicine
Kyoto University
Kyoto 606
Japan

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THE RELATIONSHIP BETWEEN THE NUCLEAR DNA CONTENT IN SMEARS OF ASPIRATES AND THE PROGNOSIS OF MUCOEPIDERMOID CARCINOMA

C-M Eneroth and A Zetterberg

From the Departments of Otolaryngology and Tumour Pathology Karolinska Sjukhuset and Institute for Medical Cell Research Karolinska Institutet Stockholm Sweden

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tract Cytophotometric analysis of nuclear DNA con nt was performed in a series of mucoepidermoid rcinomas with an extreme difference in the clinical urse which was observed during a follow-up period of 13 years The prerequisite for such a study was the velopment of a method which made it possible to study e nuclear DNA content in tumour cells obtained at the ne of diagnosis i e 6–13 years ago in the present mater l A special cytochemical procedure with destaining of e original Giemsa stain refixation and subsequent Feul n staining of the smears of aspirates in the original tological material was developed The cytophotometric clear DNA analysis of tumour cells in smears of aspi le from mucoepidermoid carcinomas showed that higher idy tumours (near triploid) had a worse prognosis when mpared with near-diploid tumours These data are sup rted by our previous findings that a shift from a near ploid to a near triploid DNA content of the tumour cell klei was associated with the prognostic important pro rty of invasive growth Thus the cytophotometric clear DNA analysis of aspirated tumour cells seems to ve valuable information about the malignancy degree of e individual tumour which is not possible to obtain only om the cytomorphological features

t has been shown that invasive salivary gland umours are characterized by a higher degree of abnormality with respect to the DNA con ent than the non invasive tumours (Eneroth & Zetterberg In press) Thus, the property of non invasive growth was found to be as sociated with a diploid or near-diploid DNA content whereas invasive growth was as sociated with a triploid or near-triploid DNA content These findings suggest that the nuclear DNA content may be a relevant cell criterion in the evaluation of the prognosis, as the invasiveness of a salivary gland tumour is supposed to be the most significant criterion in evaluating the clinical course (Blanck, 1974) The aim of the present investigation was to further study the idea about a suggested relationship between the nuclear DNA content and the prognosis of salivary gland tumours The prerequisite for such a study was to find a method in determinating the nuclear DNA content in cells of salivary gland tumours operated so long ago that the prognosis could be evaluated by a follow up period long enough The prerequisites for an analysis of the nuclear DNA content of tumours operated many years ago were present as the slides with the smears of aspirates from tumours were archived In order to be able to use this cytological material for the quantitative cytophotometric DNA analysis a special cytochemical procedure was developed

MATERIALS AND METHODS

Clinical material

Five cases were selected from a group of patients with mucoepidermoid carcinoma treated at the Department of Otolaryngology during the period 1961–1968 Three of the patients (cases 1, 2 and 3) were alive and free of symp-

toms at the end of the follow-up period (1974) 9, 13 and 6 years, respectively, after the primary operation. The remaining two patients (cases 4 and 5) died with general metastases in the tumour disease 3 years and 3 months, respectively, after the primary treatment.

Cases 2 and 3 were histologically classified as highly differentiated non-invasive mucoepidermoid carcinomas, case 1 as a moderately differentiated carcinoma without evident signs of invasive growth, and cases 4 and 5 as poorly differentiated carcinomas with general invasive growth into surrounding tissues.

Cytochemical procedures

The original air dried Giemsa stained cytological preparations, from which the primary diagnosis of the salivary gland tumours was based 5–13 years ago, were used for the cytophotometric DNA analysis. In order to be able to use this cytological material for the quantitative cytophotometric DNA analysis a special cytochemical procedure was developed, involving destaining of the original Giemsa stain, refixation and subsequent Feulgen staining. Over night treatment in absolute methanol efficiently removed most of the Giemsa stain. The small amount of Giemsa stain remaining after this procedure was completely removed during the acid Feulgen hydrolysis. After the methanol treatment the cells were refixed in 10% buffered neutral formalin for a period of 12–24 hours. This refixation proved to be important in stabilizing DNA during the acid Feulgen hydrolysis and minimize the intercellular variability in amount of Feulgen positive material. The cells were thereafter stained according to the Feulgen procedure involving acid hydrolysis at room temperature (+22°C) in 5 N HCL for the optimal time of 1 hour.

The cytophotometric measurements of stained cell nuclei were performed in a rapid scanning microspectrophotometer (Caspersson & Lomakka 1970, Lomakka 1965). Granulocytes found to be mixed with the tumour cells in each of the analysed slides were used as control cells. The nuclei from th[e] granulocytes contain DNA values correspon[d]ing to the normal diploid content (2.0 C) wit[h] variation in the majority of cells betwe[en] 1.75 C and 2.25 C. Occasionally granulocyt[es] with values up to 2.5 C were recorded. T[he] value of 2.5 C was therefore used as an upp[er] limit of the normal DNA content. The 2.5 li[mit] is indicated by the broken lines in the figu[res]. All measured values were expressed in rel[a]tion to their corresponding granulocyte co[n]trols and expressed in C units.

In order to test if any quantitative err[ors] were introduced when small, strongly lig[ht] absorbing objects were measured such as nuclei with a compact chromatin (e.g. t[he] granulocyte control cells), measurements we[re] also performed off peak at 610 nm, at whi[ch] wavelength the extinction was only about 40 [%] of that obtained at the absorption peak arou[nd] 546 nm. Independent of cell type (granulocyt[es] with a compact chromatin and tumour c[ell] nuclei with a dispersed chromatin) the ra[tio] between extinction values at 546 nm and e[x]tinction values at 610 nm was found to [be] constant. Therefore no errors of any [quan]titative significance were introduced wh[en] measuring small, strongly light absorbing ce[lls] with a compact chromatin. In addition extin[c]tion values at 546 nm above 0.5 were seld[om] recorded for the cells that had undergone t[he] above described staining procedure. T[he] representative parts of the Feulgen stain[ed] smears, i.e. the parts including the muc[o]epidermoid carcinoma cells, were select[ed] for the cytophotometric analysis and mark[ed] on the slides.

RESULTS

Five patients operated on for a parotid tum[our] histologically classified as mucoepiderm[oid] carcinoma were followed up for a period [of] 6–13 years. The five patients were select[ed] according to the clinical malignancy of th[e] tumours. In one group low grade malign[ant]

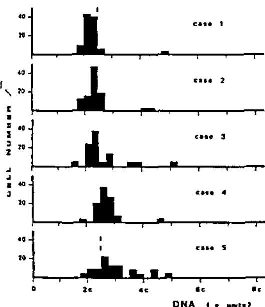


Fig. 1. Histograms of nuclear DNA content (Feulgen ... tive material) of individually analysed cell nuclei in ... rapid scanning microspectrophotometer. The cell number in each histogram class is expressed in per cent of ... total number of cells analysed. Between 50–100 tumour cell nuclei and between 10–30 granulocytes were analysed in each preparation. The measured values in each preparation were expressed in relative C-units, i.e. 2C denotes the normal diploid DNA content defined as the mean granulocyte value. Mucoepidermoid carcinomas case 1, 2 and 3 were clinical low grade malignant tumours, whereas case 4 and 5 were clinical high grade malignant tumours. The broken vertical line represents the upper extreme limit of the DNA value of ... granulocytes.

...mours were included (cases 1, 2 and 3). The patients in this group were all alive and free of symptoms at the end of the follow up period. The other group included two high grade malignant tumours (cases 4 and 5). These two patients had general metastases and died in the tumour disease 3 years and 3 months respectively after the primary treatment. Thus it could be studied whether a variability in the clinical malignancy was associated with any changes in the nuclear DNA content of the tumour cells.

From the histograms in the figure it is seen that most of the cell nuclei of the low grade malignant tumours (cases 1, 2 and 3) contained the diploid or near-diploid amount of DNA (values around 2 C). The fraction of tumour cells with abnormally increased DNA values (>2.5 C) was 9%, 25% and 34% respectively in case 1, 2 and 3. It is also evident from the histograms in the figure that the majority of cell nuclei of the high grade malignant tumours (cases 4 and 5) have DNA values corresponding to the hypo-triploid or triploid content (2.5 C to 3 C). The fraction of cells with an abnormally increased DNA value (>2.5 C) was 76% and 77% in these cases, i.e. considerably higher than in the low grade malignant tumours.

Thus, an alteration of ploidy from a diploid or near-diploid DNA content in clinical low grade malignant tumours to a triploid or near triploid DNA content in clinical high grade malignant tumours was observed.

DISCUSSION

Changes in nuclear DNA content of tumour cells have been found to be associated with the behaviour of the salivary gland tumour towards surrounding tissues (Eneroth & Zetterberg, In press). As this histological criterion (non invasive or invasive growth) has been found to be better than cellular polymorphism, mitotic rate, lymphoid stroma reaction etc. in predicting the prognosis in all types of salivary gland tumours (Blanck, 1974), it was suggested that the nuclear DNA content might be related to the prognosis of the tumour. This idea was further supported by the results of the present study indicating a relationship between nuclear DNA content of the tumour cells and their malignancy, as judged from the clinical follow up study. The present study was based on an analysis of the nuclear DNA content of tumour cells from five mucoepidermoid carcinomas selected on the basis of an extreme difference in the clinical course. Three of the patients were alive and free of symptoms at the end of the follow up period and their tumours were therefore termed low grade malignant. Two patients died in the tumour disease within 3 years, and the tumours of these patients were termed high-grade

toms at the end of the follow-up period (1974) 9, 13 and 6 years, respectively, after the primary operation The remaining two patients (cases 4 and 5) died with general metastases in the tumour disease 3 years and 3 months, respectively, after the primary treatment

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The original air dried Giemsa stained cytological preparations, from which the primary diagnosis of the salivary gland tumours was based 5–13 years ago, were used for the cytophotometric DNA analysis In order to be able to use this cytological material for the quantitative cytophotometric DNA analysis a special cytochemical procedure was developed, involving destaining of the original Giemsa stain, refixation and subsequent Feulgen staining Over night treatment in absolute methanol efficiently removed most of the Giemsa stain The small amount of Giemsa stain remaining after this procedure was completely removed during the acid Feulgen hydrolysis After the methanol treatment the cells were refixed in 10% buffered neutral formalin for a period of 12–24 hours This refixation proved to be important in stabilizing DNA during the acid Feulgen hydrolysis and minimize the inter cellular variability in amount of Feulgen positive material The cells were thereafter stained according to the Feulgen procedure involving acid hydrolysis at room temperature (+22°C) in 5 N HCL for the optimal time of 1 hour

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C M Eneroth M D
Dept of Otolaryngology
Karolinska Sjukhuset
S 10401 Stockholm
Sweden

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GOBLET CELLS IN THE DEVELOPING HUMAN NOSE

J Poulsen and M Tos

From the ENT University Clinics Rigshospitalet and the Gentofte Hosp tal Copenhagen Denmark

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Abstract In a material of 56 foetuses and prematures in the 9th to the 30th week the nasal mucosa was removed *in toto* and stained with PAS–alcian blue PAS and the osmium whole mount methods The development spread distribution and density of goblet cells were studied Goblet cells start forming anteriorly in the nasal vestibule in the 13th week spreading backwards according to a given constant pattern in the course of the subsequent week By the 30th week the goblet cells are present throughout the respiratory region but there are marked differences in density between the various areas The highest density was found anteriorly and inferiorly on the septum and lateral wall and also in the inferior and middle meatuses On the conchae the density is less at the junction to the olfactory region least As a whole the density is still very low in foetuses and prematures and it must increase very considerably around the time of birth The differences in density found in the present study are probably present also to some extent in children and adults Formation of goblet cells is the final link in the process of differentiation into respiratory epithelium instituted 4 weeks earlier with the formation of ciliated cells

It is well known that the epithelium of the respiratory region of the nose contains goblet cells but nothing definite is known about their distribution or density in the various parts of the nose Together with the seromucous glands of the nose the goblet cells produce the nasal secretion which plays such an important role in the physiology and pathology of the nose However any changes in goblet-cell density which may occur under pathological conditions—often described (Hajek, 1905, Oppikofer, 1907, Hansel, 1930, Eggston & Wolff, 1947, Messerklinger, 1958, Mygind et al , 1974) can be accurately assessed only when their density in the normal muc known

The object of the present study v ascertain the spread and distribution of cells during the development and growth nose This ought to form a basis for stuc the distribution in children and adults endeavoured to ascertain whether diffe in density between the various regions present already in foetuses and prematu order later to be able to interpret gobl density in children and adults There been no special studies of the occurre goblet cells in foetal noses and no quant studies of their density in children and According to Clara (1938) the differen from embryonic to pseudostratified c epithelium has been completed by th months Bang (1964) finding goblet anteriorly in the nose in the 11th wee lieved that they appeared before the m glands In the 17th week she found a density of goblet cells anteriorly in the

MATERIAL AND METHODS

The material comprises 56 foetuses an matures in the age range 9th to 30th men week, assessed on the basis of crown- length (CR) and foot length according to ter's tables (1921) After fixation and d tion from the outside, separating the nasa

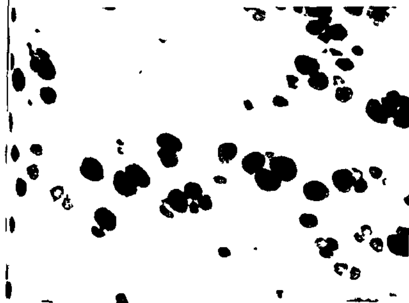


Fig 1 Clustering of goblet cells Several twin cells Septum in 17th week PAS–alcian blue whole mount, ×350

osa *in toto* from the surrounding tissue, one alf of the nose was stained by a PAS-alcian ue whole mount method The goblet cells ained a bright blue on a pale blue back- [1] The other half of the nose was stained ither by the PAS whole-mount method ich stained the goblet cells red, or by the im tetroxide whole-mount method which oes not stain the goblet cells, but the mucous lands This latter method affords great help in differentiating between groups of goblet cells and young gland primordia in the youngest foetuses The advantages and disadvantages of these methods as well as the fine dissection at various ages have been described previously (Tos, 1966, 1970)

In each nasal half the spread of goblet cells was determined The density was divided into three grades High, moderate, and low, and the spread of the three density zones was



Fig 2 High density of goblet cells anteriorly on the septum in 16th week with marked PAS–alcian blue whole mount ×60

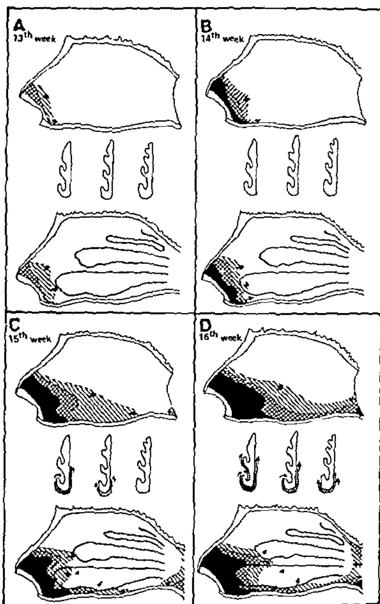


Fig 3 Spread of goblet cells at dif ages *Top* Septum in the centre three tal sections through the anterior pole dle and posterior pole of the middle cha *Bottom* Lateral wall with the inf middle superior and supreme co Zones with high density black with m ate density heavily shaded and with density lightly shaded Arrows indica rection of spread dotted arrows the tion beneath the conchae

traced on a graph for each foetus On six foetuses representing different age groups, the density was determined quantitatively, and the goblet cells were counted in Reichardt's projection microscope in a magnification of 500× According to foetal age and goblet cell spread, the counts were made in 100–230 fields measuring 0 01768 mm² equally distributed on the three density zones and the median density for each zone in the various age groups, was calculated Thereafter, parts of the whole mounts studied were cut into serial sections, stained with haematoxylin eosin and investigated

RESULTS

In the youngest foetuses, 9th and 10th weel which goblet cells had not yet formed, epithelium was in the course of differentia into pseudostratified ciliated Anteriorly it tall, pseudostratified, columnar with nur ous ciliated cells, posteriorly shorter v fewer ciliated cells and hardly as mature as teriorly This indicates that ciliated cells fi before the goblet cells and that the proces epithelial differentiation starts anteriorly in nose and proceeds in the anteroposterior rection



Fig 4 Anterior half of nasal septum in 15th week ...ters of goblet cells anteriorly in the vestibule Mucous ...ds have spread farther back with the goblet cells ...S-alcian blue whole mount ×30

Appearance of goblet cells is the final link in ...e process of differentiation of the respiratory ...ithelium In a few cells mucin granula form ...d gradually increase in quantity In whole ...unts this process is visible in the form of ...all faintly PAS alcian blue positive spots ...ich gradually increase in size and staining ...ensity The goblet cells are especially in ...e younger foetuses as a rule in clusters of ...o (twin cells) four or more (Figs 1 and 2) ...his grouping may mean that mitotic division ...f the goblet cells can take place The foetal ...oblet cells are large distended with mucin

...pread of goblet cells

...oblet cells start forming in the 13th menstrual ...eek in a zone most anteriorly in the nasal ...avity (Fig 3) and spread in the course of the 14th 15th and 16th week backward espe...cially along the nasal floor Hence they spread upwards both on the septum (Fig 4) and be...neath the inferior concha (Fig 5) From the rhinopharynx and the soft palate the goblet cells situated mainly in the mucosal sulci force their way towards the nasal cavity, to join the anterior goblet-cell front on the nasal floor in the 16th week

In the 17th week (Fig 6A) the goblet cell zone on the septum extends upwards (Fig 7) on the lateral wall up into the inferior meatus and backwards to the anterior third of the middle meatus and the medial surface of the inferior concha By the 19th week (Fig 6B) the entire inferior meatus is lined with goblet cells From the middle meatus they have spread up on the lateral surface of the middle concha and down over the anterior third of the medial surface of the inferior concha From the rhinopharynx the goblet cells have spread forward in the posterior quarter of the middle meatus Hence their spread continues down over the posterior pole of the inferior concha and up beneath the middle concha and further down on its lateral surface lower edge and medial surface

By the 23rd week (Fig 6C) the entire in ferior concha and middle meatus have also become lined with goblet cells From the en tire extent of the middle meatus the goblet cells spread out on the lateral surface of the



Fig 5 Anterior half of the lateral wall in 15th week The goblet cells reach to the anterior pole of the inferior con cha the glands farther back PAS–alcian blue whole mount ×30

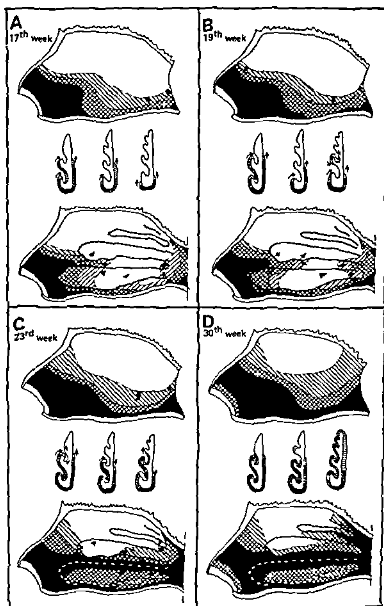


Fig 6 Spread of goblet cells at [illegible] ages Explanation as in Fig 3

middle concha, down towards its lower edge, and up on the posterior half of its medial surface Furthermore, an extension spreads *from the rhinopharynx up towards the* supreme concha, onwards in the superior meatus and hence up beneath the superior concha and down over the medial surface of the middle concha Anteriorly the goblet cells spread over *the anterior pole of the middle* concha On the septum the goblet-cell front reaches anteriorly almost to the nasal roof and posteriorly to the upper edge of the choanae

By the 30th week there are goblet [illegible] throughout the respiratory region whose [illegible] to the olfactory region, which has no [illegible] cells, may be seen in Fig 6D The bou[illegible] runs on the middle concha in such a wa[illegible] the entire posterior half and lower third [illegible] medial surface, as well as the entire [illegible] surface, are lined with goblet cells More[illegible] the posterior two thirds of the superior m[illegible] and posterior third of the superior conc[illegible] well as the entire supreme concha are [illegible] with goblet cells



7 Anterior half of septum in 17th week High goblet density in the lower half none in the upper PAS–al blue whole mount ×30

...sity of Goblet Cells

...s 3 and 6 show the distribution and spread the three density zones in the course development Fig 8 the corresponding ...ntitative measurements for each zone and ...h age group Changes in density are best ...trated on the nasal septum After the first ...let cells have formed anteriorly on the ...tum, the density in this area increases ...dually and regularly during the subsequent ...r 3 weeks (Fig 3), so that by the 16th week ...re is a fairly great density in the anterior ...rd of the respiratory region (Figs 2, 3 D ...d 8) After the goblet cells have spread dur... the subsequent weeks, to the posterior and ...erior areas of the septum (Fig 6) the den...ty in these sites also increases gradually, ...t not as markedly and not as rapidly as in the ...terior parts In the course of 7 weeks—from ...e 23rd (Fig 6 C) to the 30th week (Fig ...D)—the border marking high density has not ...oved essentially upwards or backwards, and ...is probable that at birth there is a considera...ly lower density in the upper part of the ...eptum, especially at the limit to the olfactory ...egion than in its lower part

Similar appearances apply to the lateral wall, only far more complicated because of the conchae In the anterior part the density increases, regularly and rapidly (Figs 3 and 8), and later it increases also in the inferior and middle meatuses, but not nearly as much as anteriorly, and especially not on the conchae, where it is still not particularly high in the 30th week (Fig 6 D) It is low also at the junction to the olfactory region, and at birth it is probably lower here and on the conchae than in the meatuses

The following factors influence the goblet cell density during the development of the nose (1) The time of the first appearance of goblet cells, as the density is on the whole higher anteriorly, where goblet cells form first, than posteriorly (2) Regional differences in density Anteriorly on the inferior concha, superiorly in the nasal vestibule, and infero-posteriorly on the septum, where the first goblet cells appear as early as the 14th and 15th weeks (Figs 3 B and C) density is still relatively low in the 30th weeks (Figs 6 D) During this period, the density in these regions did not increase to the same extent as in other regions, and possibly it is also lower after birth in these regions (3) Growth of the mucosa, whose area is constantly increasing To keep pace with the growth of the area and maintain the attained density, new goblet cells have to form constantly During the first weeks a rela

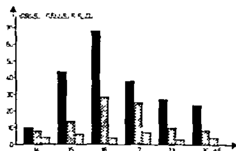


Fig 8 Median density of goblet cells (number/0 01768 mm² field) at different ages and different density zones the extent of which is illustrated in Figs 3 and 6 Black heavily and lightly shaded columns represent density zones showing high moderate and low density respectively

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J Poulsen M D
ENT University Clinic
Rigshospitalet
9 Blegdamsvej
DK 2100 Copenhagen
Denmark

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THE PATENCY OF THE MAXILLARY OSTIUM IN RELATION TO BODY POSTURE

R Aust and B Drettner

From the Department of Otolaryngology University Hospital Uppsala Sweden

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stract Eleven subjects were investigated concerning e effect of postural changes on the functional size of the aullary ostium The ostial size expressed as the centage of the initial value decreased only slightly en the body position was altered from 90° (sitting) to ° while the decrease between 30° and 0° (lying) was re pronounced A cuff around the neck inflated to 25 mHg gave a decrease corresponding to a value of 20° dy position Insufficient ventilation of the paranasal uses when lying down may result especially in cases eady having small ostia in the sitting position

itent ostia of the paranasal sinuses are essen-l for their normal functioning Obstructions , for example, the maxillary ostium can be e first step in a process leading to an acute nusitis However, even when the ostium is itent the specific size is significant When e ostium has a diameter less than 2 5 mm, dicating a relative ventilation insufficiency, e oxygen content of the maxillary sinus is wer than normal (Aust & Drettner, 1974) It therefore of interest not only to evaluate the itency of the maxillary ostium, but also its nctional size under various conditions

One of the factors which may influence the nctional size of the maxillary ostium is body osture Winslow et al (1934) found an in reased swelling of the nasal mucosa in the ecumbent position Rundcrantz (1969) reported that the nasal airway resistance increased in positions of 20° or less above the horizontal plane This was found in healthy subjects as well as in patients with allergic and infectious rhinitis, although more accentuated in the latter patients An increased airway resistance was also found during compression of the neck Jonson & Rundcrantz (1969) showed that compression of the neck with 25 mmHg gave the same increase in pressure within the bulb of the internal jugular vein as the change in body position from sitting to recumbent A change in the patency of the Eustachian tube according to posture was also found (Rundcrantz, 1969)

No studies of postural changes of patency of the maxillary ostium have been reported in the literature Investigation must in the first place concern normal subjects, since about 50% of persons with allergic or infectious rhinitis already have obstructed ostia in the sitting or semi-recumbent position (Drettner & Lindholm, 1967)

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MATERIAL AND METHODS

Twelve subjects (9 men and 3 women) in the age range 20–32 were investigated Eleven of them were healthy with no signs of allergy or infection and had normal rhinoscopy and radiographs of the paranasal sinuses One sub-

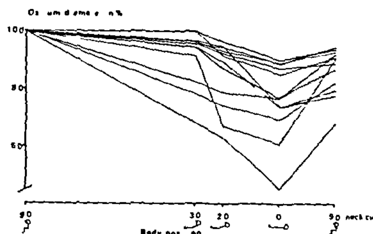


Fig 1 The diameter of the maxillary ostium in different body positions presented as a percentage of functional size in the erect body position Values obtained in 11 subjects with the mean illustrated by an interrupted line

ject had a common cold with slight mucosal swelling of the maxillary sinus as revealed radiographically One of the maxillary sinuses usually the right was punctured with a cannula of 2 mm diameter The puncture was performed in the inferior meatus after local anaesthesia with a Xylocain® spray without adrenalin

The patency of the maxillary ostium was checked by pressure recordings from the sinus during breathing blowing and sniffing (Drettner 1965) when the patient was lying with the upper part of the body inclined 45° above the horizontal plane When still lying in the same position another cannula was introduced in the same maxillary sinus close to the first and its patency was also checked in the same way as the first

The functional size of the maxillary ostium was measured according to a principle published recently (Aust & Drettner 1974) One of the cannulas was used for intermittent insufflation of an airflow of 1 2 or 4 l/min The other cannula was used for recording the increase in pressure when the airstream entered via the sinus and left via the ostium Calibration using the same cannula size was performed in a model in which the outlet representing the maxillary ostium was a tube with a length of 6 mm and varying diameters The results were shown to be unrelated to the size of the maxillary sinus (Aust & Drettner 1974) The pressure increase obtained in the subject was analysed using a nomogram from model experiments In this way the functional size of the maxillary ostia in the subjects could be calculated

The subjects were first investigated with the upper part of the body perpendicular to the horizontal plane After a half minute in erect the same procedure was performed in positions 30° and 20° above the horizontal and then lying horizontally Finally in an erect sitting position the neck was compressed by an inflatable cuff The pressure in the cuff was mmHg (35 cmH₂O) which was the same pressure used by Rundcrantz (1969)

RESULTS

The subject with a common cold had an obstructed ostium during ordinary breathing and its size could therefore not be measured The other subjects all had patent ostia Their functional size decreased when the body position was changed from a sitting to recumbent position Fig 1 shows the changes in the diameter of the functional size of the ostium expressed as percentages of the original diameter The mean values were 95% at 30° 87% at 20° and 77% at 0° (=recumbent position) The corresponding value at 90° (=erect position) with a cuff inflated to 25 mmHg around the neck was 87% of the original size i e similar to the values measured at 20° body position

The mean functional diameter of the ostium

able I *The functional size of the maxillary ostium—expressed as mm diameter and mm² cross ction area—in 11 subjects investigated in different body positions and with a neck cuff inflated 75 mmHg The volume of the maxillary sinus measured roentgenologically is also presented*

		Maxillary sinus volume	Functional ostium diameter in mm					Ostium cross section area in mm²				
I	Age	(ml)	90°	30°	20°	0°	90°+neck cuff	90°	30°	20°	0°	90°+neck cuff
	27	15 5	1 1	0 8	0 7	0 5	0 8	1 0	0 4	0 4	0 2	0 5
	31	10 1	1 1	1 1	1 1	1 0	1 1	1 0	1 0	0 9	0 8	0 9
	29	7 8	1 8	–	1 7	1 6	1 7	2 5	–	2 3	2 0	2 3
	2?	27 3	1 9	1 8	1 7	1 4	1 5	2 8	2 5	2 3	1 5	1 7
	23	20 2	2 3	2 1	2 1	2 0	2 0	4 0	3 6	3 4	3 0	3 1
	27	24 9	2 3	1 8	1 7	1 6	1 9	4 2	2 5	2 3	2 0	2 8
	20	13 8	2 4	2 2	1 6	1 4	2 2	4 3	3 6	1 9	1 6	3 6
	21	27 5	2 6	2 2	2 1	2 0	2 4	5 3	3 6	3 3	3 1	4 5
	27	24 9	2 8	2 7	2 6	2 5	2 6	6 2	5 7	5 3	4 9	5 3
	25	26 2	3 4	3 4	3 1	2 9	3 1	9 2	9 2	7 6	6 6	7 6
	32	23 5	5 6	5 4	5 3	4 4	4 8	24 6	22 9	22 1	15 2	18 1
n	25 8	20 2	2 5	2 4	2 2	1 9	2 2	4 8	4 3	3 6	2 9	3 7

e erect position was 2 5 mm (cross section 4 8 mm²) and the corresponding value in recumbent position was 1 9 mm (2 9 mm²), ch was also the lowest mean value (Table easured in any position The number of ects who had an ostial diameter smaller 2 5 mm was 7 in the erect position and 8 e recumbent position

hus only one subject who had an initial l diameter greater than 2 5 mm in the t position had values below 2 5 mm in the e recumbent position However, most of subjects had values smaller than 2 5 mm n in the erect position

DISCUSSION

ce injection of air into the paranasal sinuses usually considered dangerous it is worth essing that the precautions against acci its were rigorous After introduction of ch cannula into the maxillary sinus, pressure cordings were performed to ensure that the nnula was not blocked and that the maxil ry ostium was patent The insufflation of air as performed for only a few seconds each ne and the airflow was chosen to be as small possible No complications or discomfort ere experienced during the air insufflation

The mean size of the maxillary ostium in the present study (2 5 mm) was exactly the same as in a larger series (Aust & Drettner, 1974) and the series seems therefore to be rep resentative for a normal population

The present procedures for changes in body position and for neck compression were essentially the same as those used by Rundcrantz (1969) Thus, it was possible to compare the results concerning changes in the maxillary ostium with changes in the nasal patency and Eustachian tube function It is also obvious that all such postural changes are caused by hydrostatic changes in the venous pressure Patients with allergic or infectious rhinitis were excluded from the present study since they usually have obstructed ostia of the maxillary sinus The changes in the size of the maxillary ostium in the present investigation were very similar to those reported by Rundcrantz (1969) concerning nasal patency Thus both the changes in nasal patency and in ostial size were less between 90° and 30° than between 30° and 0° The compression of the veins of the neck with a cuff having a pressure of 25 mmHg resulted in a change in ostial size which corresponded to that occurring when the position was altered from sitting to corresponding effect on nasal

compression was still closer to the horizontal position, according to Rundcrantz (1969)

When the measurements of the maxillary ostial size are compared with the investigations of the function of the Eustachian tube (Rundcrantz, 1969) it is obvious that the patency of the tube decreased considerably, sometimes to such an extent that the ventilatory capacity became insufficient when the patient changed from the erect to recumbent position A similar tendency was found concerning the maxillary ostium, though remaining patent in all subjects who originally had patent ostia

A functional ostial diameter of 2 5 mm is usually required for sustaining a normal oxygen level in the maxillary sinus It is therefore of interest that only 4 of 11 subjects had such an ostial size in the erect position while in the recumbent position only 3 subjects maintained such a size If these figures are representative of the whole population, it is obvious that insufficient ventilation of the sinus, with a tendency towards a decreased oxygen content is very common The distribution of the diameters was large in the present series but it was similar to that in a recently published series comprising 37 cases (Aust & Drettner, 1974) Many apparently normal subjects thus have an ostial diameter which may be more or less insufficient for the maintenance of the antral oxygen content at a normal level So far, no conclusive evidence can be presented for a direct correlation between low oxygen content and sinusitis Still it is worth noting that an anatomical physiological disorder of the ostia of paranasal sinuses may be a predisposing factor for sinusitis

The present patient population was primarily composed of normal subjects It seems likely that the postural swelling of the mucosa in the maxillary ostium shown in normal subjects, may be at least as pronounced in patients with infectious or allergic rhinitis Thus the frequent occurrence of obstructed ostia even in the erect position in these patients may be still greater in the recumbent position and the ostial resistance may also be more pronounced among those who even in the erect position have obstructed ostia of the maxillary sinus or some other paranasal sinus

Since the oxygen tension in the maxillary sinus is not only decreased when the ostium is obstructed but also influenced by the degree of obstruction (Aust & Drettner, 1974) such an increase in the resistance of the obstruction of the ostium during bed rest may have a harmful effect, especially during an upper respiratory infection

ZUSAMMENFASSUNG

Elf Probanden wurden im Hinblick auf posturale Grössenveranderungen der Maxillarostien untersucht Der Ostiumdurchmesser veranderte sich im Vergleich zum initialen Wert nur geringfugig bei Lageanderung der Probanden von 90° (sitzend) zu 30° dagegen fanden wir *eine deutliche Verengung im Bereich von* 30° bis (liegend) Beim aufrecht sitzenden Probanden ergab eine am Hals angelegte mit 25 mmHg aufgeblasene Gummimanschette die gleiche Ostienverengung wie eine Lageanderung von 90° auf 20°

Insuffiziente Ventilation der Nasennebenhöhlen bei liegenden Probanden kann auf Ostien zuruckzuführen sein die bereits in aufrechter Position einen geringen Durchmesser aufweisen

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R Aust M D
Dept of Otolaryngology
University Hospital
S 750 14 Uppsala 14
Sweden

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OLFACTORY NEUROBLASTOMA

C G Lindstrom and D W Lindstrom

From the University Department of Pathology the General Hospital Malmo and ENT Department Hospital of Angelholm Angelholm Sweden

(Received December 7 1974)

ict A case of olfactory neuroblastoma type I ory neurocytoma) in a 44 year-old man is de d The tumour grew extensively in the right nasal with involvement of the maxillary sinus and eth l region but was radically removed at operation ognosis and treatment are discussed in the light of literature

tory neuroblastoma is an uncommon ur originating in the neuroepithelium of lfactory area More than 100 cases have reported The tumour is malignant and to metastasize and is sometimes fatal

REPORT OF CASE

patient is a 44 year old electrician Some s previously he had had a minor traffic lent and roentgen examination at that sion had revealed a density in the right illary sinus The finding was interpreted as norrhage in the maxillary sinus following accident No fracture was demonstrable ortunately none of the roentgenograms n on that occasion are available It is not kely that the density in the maxillary sinus in reality due to the tumour to be de bed Repeat roentgenography was not e to check the finding The patient had erwise always felt well apart from an occa ial cold During the last year he has been ibled more and more by colds and nasal ech In May 1972 he was referred to examination because of nasal obstruction and symptoms resembling those of right sided otosalpingitis The roentgen diagnosis was right sided pansinusitis without fluid levels and the patient was treated with antibiotics and nasal drops The patient refused irrigation of the maxillary sinus

At follow up some weeks later the patient no longer had any acute symptoms but the roentgenogram was of the same appearance as before (Fig 1) Rhinoscopy now revealed a small polyp like formation in the right side of the nose, a formation which had previously escaped detection because of the swelling of the nasal mucosa At evulsion the tumour was felt firmer than expected from an ordinary nasal polyp Histological examination of the specimen showed the picture of olfactory neuroblastoma

The patient was therefore admitted for further examination Skull X ray showed no intracranial changes and no osseous altera tions suggesting involvement of the dura To mography of the nasal sinuses revealed de struction of the medial wall of the right maxil lary sinus and persistence of the massive cloudiness of the sinuses on the right side The sphenoidal sinus and the frontal sinus had a n rmal air-content Right sided carotid angio gr phy did not show any changes in itracranial vessels Ophthalmologic neurologic examination revealed no



Fig 4 Detailed picture olfactory neuroblastom (type I olfactory neuro cytoma) with regular ce often arranged in rows H & E ×184

The most common clinical symptoms are unilateral nasal obstruction, epistaxis, bulging of the tumour (presenting mass), pain and headache and excessive lacrimation (Skolnik et al , 1966)

It must be differentiated from all other forms of tumour of the upper part of the nasal sinus and the ethmoidal region and from lymphosarcoma and small-celled undifferentia carcinoma (Obert et al , 1960) as well as fr the rare forms of tumour such as extracrar meningioma (Lindstrom & Lindstrom 1969

The radiosensitivity of olfactory neu blastoma has probably been over-estimated the earlier literature These tumours are oft not very radiosensitive (Gerard Merchant

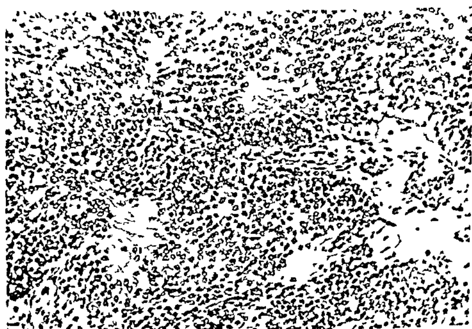


Fig 5 Part of olfacto neuroblastoma (type I olfactory neurocyt ... cells arranged in para converging rows and f chromatin network ... nuclei Networks of ne fibrils between the ce H & E ×1...

...heau, 1965; Fitz-Hugh et al., 1965) and this ...es particularly to neurocytoma (Grahne, ...

Combined surgery and pre- or post-...tive radiotherapy have been tried, but re-...nces have nevertheless appeared in about ...f the cases. It is still too early to say any-... about the value of cytostatic therapy. On ...asis of the 5-year-survival rate Skolnik ... (1966) recommend surgery of the pri-... lesion and reservation of radiotherapy ...ned with surgery for recurrences. In the ...nt case treatment was planned according ... principle.

ZUSAMMENFASSUNG

...der Fall eines Olfactorius Neuroblastoms Typ I ...nus Neurozytom) in der Nasenhohle eines ...en Mannes beschrieben Weder klinisch noch ...ologisch konnte der Nachweis erbracht werden, ...r Tumor intrakraniell wirksam war Der Tumor ... durch Radikaloperation entfernt, es erfolgte ...1 zweijahriger Observation kein Rezidiv Die ...kation und die Therapie dieser seltenen Tumoren ... diskutiert

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C Lindstrom M D
Dept of Pathology
General Hospital
S 214 01 Malmö
Sweden

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MUCOSAL MALIGNANT MELANOMAS OF THE HEAD AND NECK

With Special Reference to Cases having a Prolonged Clinical Course

C -M Eneroth and C Lundberg

From the Departments of Otolaryngology and Tumour Pathology Karolinska Sjukhuset Stockholm Sweden

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Abstract A histological re-examination and re-classification of primary mucosal tumours of the head and neck region treated at Radiumhemmet and Karolinska sjukhuset during the period 1927–1970 revealed that 41 tumours were malignant melanomas All these 41 tumours were located in the nasal cavity paranasal sinuses and oral cavity and not a single case of primary mucosal malignant melanoma was found in other locations of the head and neck region In the present study the long term prognosis has been analysed The follow up period was at least 5 years and ranged up to 48 years It was found that mucosal malignant melanomas had a very poor prognosis with a five year survival rate of 17 per cent (7 of the total 41 cases) and a ten year survival rate of 7 per cent (3 of the total of 41 cases) The unpredictability of the clinical behaviour of this tumour type is elucidated by cases with a prolonged clinical course despite a primary relatively limited surgery repeated local recurrences and regional lymph node metastases in an early stage of the disease Thus there is always a never-ceasing risk of death in the tumour disease when once a malignant melanoma has occurred For this reason a meticulous and long follow up of tumour patients is stressed and also the value of repeated surgery of local recurrences and regional lymph node metastases

Although malignant melanoma is regarded as a highly malignant type of tumour, the unpredictability of its clinical behaviour has been pointed out Thus cases of primary cutaneous malignant melanoma with a prolonged clinical course, despite regional lymph node metastases already at an early stage of the disease have been reported However, mucosal malignant melanomas are found to carry a much poorer prognosis than cutaneous malignant melanomas (Allen & Spitz 1953, Moore & Martin 1955, Catlin 1967 Anneroth et al , 1973) Thus the mean survival time [is] shorter in mucosal than in cutaneo[us] malignant melanomas, which can explain w[hy] reported cases of mucosal maligna[nt] melanomas with a prolonged clinical cour[se] (>10 years) cannot be traced in the literatur[e] with few exceptions (Catlin 1967, Freedma[n] et al , 1973) In order to study the prognosis [of] mucosal malignant melanomas of the head an[d] neck and to establish whether any of the[se] tumours had a prolonged clinical course a[n] extensive tumour material with a very lo[ng] follow up period (5–48 years) was analysed

MATERIAL AND METHODS

The present study is based on 41 patients w[ith] primary mucosal malignant melanomas in t[he] head and neck region treated at Radiu[m]hemmet and the Department of O[to]laryngology Karolinska sjukhuset du[ring the] period 1927–1970 In 24 of the 41 cases [the] primary malignant melanoma was located [in] the nasal cavity and paranasal sinuses and [in] the remaining 17 cases in the oral cavity Du[r]ing the period 1927–1970 not a single case [of] primary malignant melanoma was found [in] mucosal locations of the head and neck reg[ion] other than those mentioned above ev[en] though a few cases with metastatic deposi[ts] from primary malignant cutaneous melanom[as] were found in the mucous membranes of t[he]

ynx, larynx and trachea. Of the 24 cases ere the tumour involved the nasal cavity, 8 was involved both the nasal cavity and s of the paranasal sinuses, whereas 16 e limited to the nasal cavity without affect- the paranasal sinuses. In 8 of the 17 pa- s with malignant melanomas in the oral ty, the tumour was located in the palate, in another 8 cases in the superior alveolus, reas in one case the tumour was located in buccal mucosa.

he clinical follow-up study comprises all 41 patients operated upon during -1970 and continued until 31 January, The postoperative follow-up examina- of the patients was made at least once ly, both at Radiumhemmet and their local itals, and the data on the clinical course noted in the case records at Radium- met. The observation period (5–48 years) s from the first histological verification of umour.

e prerequisites for a histological re-classi- on exist, as the entire tumour material is le at the Institute of Tumour Pathology, olinska sjukhuset, partly as sectioned imens and partly as paraffin-embedded rial. The histological re-classification of material was made according to the criteria alignant melanoma described by Eneroth oberger (1973) and Anneroth et al. (1973).[1] all 41 cases, the histological re-examina shows definite malignant features with ly differentiated cells, often with a great ber of mitotic figures. In those cases re the tissues surrounding the tumour ld be analysed, the tumour cells showed an nsive and diffuse infiltrative growth into e tissues, whereas the plasmo-lymphatic reaction with single exceptions were mar- ly sparse. The histological diagnosis was ed on the presence of melanin pigment and structure of the cells. Essentially three es of cell structure occurred. One was composed of spindled, elongated, fusiform, usually richly pigmented cells of fibro-sarcoma-like structures (19 cases), another of large, usually rounded polymorphous undif-ferentiated and poorly pigmented cells of car-cinoma-like structures (14 cases), and the third a mixture of the fibrosarcoma-like and car-cinoma-like types (8 cases).

[1] he histological reclassification was checked by G. berger, Professor at the Institute of Tumour Patholo- Karolinska sjukhuset.

CLINICAL FINDINGS

Mucosal malignant melanoma of the head and neck region had no sex predilection (24 men and 17 women) in the series, and the age at the primary diagnosis of the tumour ranged from 34 to 80 years (mean 60 years). The symptoms varied with the site of the tumour and will not be discussed further. The duration of the symptoms before medical attention varied be-tween one month and five years (mean 9 months). During the whole follow-up period local recurrences appeared in 21 of the 41 cases, and the first local recurrence occurred in all 21 cases within five years of onset.

Metastases

Metastases appeared in 38 of 41 cases (93%) during the whole follow-up period. In 16 of the 41 cases metastases were demonstrable pre-operatively or at the first treatment.

In all these 16 patients the metastases were located in the regional lymph nodes, i.e. there was no case of distant metastases diagnosed at the time of the first surgical intervention. Re-gional lymph node metastases appeared during the follow-up period in another 11 of the 41 cases, and all 11 cases within a period of 5 years from the first histological verification of the tumour (5 cases within 1 year, 4 cases between 1–3 years and 2 cases between 3–5 years).

Distant metastases appeared in 23 of the 41 patients after the primary treatment. The in-terval between the first operation of the tumour and the appearance of metastases is seen in Table I. Further, this table shows in all but 7 cases metastases appeared wi years of the first operation. In 4 ca

Table I Primary mucosal malignant melanomas of the head and neck Time interval between the first histological verification of the tumour and the appearance of distant metastases in 23 cases

Time interval (years)	Number of patients
<1	10
1–3	6
3–5	3
>5	4
Total	23

distant metastases did not appear until after 5, 5½, 14 and 32 years, respectively The two patients with the first appearance of distant metastases 14 and 32 years after the initial treatment of the tumour are unique, and the case history of these two patients will be given in detail (See case reports)

Survival

Thirty-nine of the 41 patients died during the follow up period (Table II) It is evident from Table II that only 4 of the 41 patients did not die of the tumour disease during the follow-up period One of the two patients registered as having died of intercurrent disease committed suicide seven months after the treatment of the primary tumour, but distant metastases had already developed The other patient in this group died 4 years after the first surgical intervention from a cardiac infarction without any signs of the tumour disease The two patients noted as living free of tumour disease were women and both were 34 years old at the initial treatment—one of them was living free of the tumour disease 16 years after radical operation of a malignant melanoma of the superior alveolar process, whereas the other patient was living free of the tumour disease 5 years after the operation of a mucosal malignant melanoma of the nasal cavity

The survival time of the 37 patients who died in the tumour disease during the follow-up period is seen in Table III It is obvious that the survival time after the initial treatment is short Thus, most of the patients had di[ed] within 3 years, and all but 5 patients within [...] years

As the main purpose of the present stu[dy] was to analyse cases of mucosal maligna[nt] melanomas with a prolonged clinical cou[rse] the two patients with a survival time of [more] than 10 years will be described in detail as ca[se] reports

CASE REPORTS

Case I

A 62-year-old woman was admitted to Kar[o]linska sjukhuset in 1959, with the chief com[plaint] of recurrent nosebleeds from the r[ight] nostril for two months Examination reveal[ed] a soft hemangioma like tumour mass w[ithin] the upper part of the right nasal cavity [It] appeared to have originated from the muco[sa] of the septum A biopsy was made and r[e]ported a malignant melanoma Therefore [a] wide local cautary excision of the tumour wa[s] made About one year after the primary exc[i]sion regional lymph node metastasis appeare[d] at the left side of the neck and therefore a le[ft] neck dissection was performed The histolog[i]cal investigation established metastatic de[po]sits of malignant melanoma in the lymp[h] nodes The patient did well until 1964 when [a] black coloured local recurrence was found and the patient underwent a Denker operation of the left maxillary sinus with trans-antr[al] ethmoidectomy and sphenotomy It was foun[d] that the tumour had involved the bone so tha[t] the dura was laid bare After this operation the patient was free from symptoms for thre[e]

Table II A follow up study for 5–48 yea[rs] of 41 patients with primary malignant mucos[al] melanomas of the head and neck

	Died of		Living	
Number of patients	Tumour disease	Inter current disease	Free of tumour	With tumour present
41	37	2	2	-

able III *Primary mucosal malignant melan nas of the head and neck Survival time er first histological verification of the no r in 37 patients who died of the tumour ease*

al time ars)	Number of patients
	12
	15
	5
0	3
	2
l	37

rs but in 1967 a large greyish red mass d the nasal cavity and was found to be ted mainly on the lateral wall of the right l cavity A Denker operation of the right illary sinus with trans antral eth lectomy was performed After this opera the patient had a very wide nasal cavity as septum and the lateral walls of the nasal ty were extirpated and the ethmoidal ses were evacuated bilaterally After this ation there was no evidence of the tumour ase until 1969 when a local recurrence extirpated During the period 1969–1973 sion and electricautery of 11 local recur es were performed The local recurrences e small and of different colours such as red brown or black and were almost ays preceded by small bleedings from the e The patient did quite well during this od and the only trouble was crusts in the al cavity During 1973 the local recur ces appeared more frequently than before were also more extensive In September 3 the first manifestation of distant astasis appeared in the form of a very big our in the small intestine which was resec

In December 1973 a metastasis in the illitium was extirpated and in February 74 the patient was admitted to hospital with heavy nosebleed The whole nasal cavity s filled with a large grey blue bleeding nour mass and on this occasion metastases the mammary region were detected

Thus after a 14 year period relatively tranquil tumour disease suddenly changed character and could not be controlled by further surgical activity The patient died in April 1974 in the tumour disease—15 years after the first histological verification of the tumour

Case 2

A 37 year old man about three years before admission to his local hospital (1936) noticed a strip of brown discolouration of the upper alveolar mucosa The brown patch had not bothered him so he ignored it for three years (until 1936) although it slowly enlarged At this time he noticed a discolouration in the mucosa of the upper lip and the first examination of the patient revealed besides a non-elevated pigmented area (2×3 cm) in the upper alveolar mucosa and an indurated brown pigmented area (1×1 cm) in the mucosa of the upper lip to the right of the midline No cervical lymph nodes were palpable During the period 1936–1939 the pigmented areas were twice excised but unfortunately pathology reports are not available Not until 1939 did a histological examination reveal that a pigmented tumour excised from the upper lip was a malignant melanoma In 1940 the patient was admitted to Radiumhemmet and the physical examination revealed a blue discoloured scar in the mucosa of the upper lip and an irregular firm brown pigmented tumour on the upper alveolar mucosa The tumour caused the patient no discomfort except for bleeding at the slightest provocation A wide cautery excision of the tumour with the surrounding bone walls of the superior alveolar process and the pigmented area of the upper lip was performed In 1941 neck dissection was performed on the right side and in 1942 on the left side Metastatic deposits of malignant melanoma were found in regional lymph nodes in the specimens from both sides After cautery excision and irradiation of a local recurrence in the upper 1942 the patient was free of sy tumour disease until 1966 (24 y

neck metastasis was extirpated on the right side, and the histological examination revealed malignant melanoma Postoperatively the right area of the neck was irradiated with a total dose of 6000 r Two years later (1968) cervical lymph nodes were palpable in the supraclavicular region bilaterally and distant metastases appeared in the lungs The patient died in August 1968 in his tumour disease 35 years after the first symptoms of the disease

TREATMENT

The 41 patients with mucosal malignant melanomas were treated with surgery, radiation therapy and chemotherapy in varying combinations It is obvious of course, that the treatment could not have been uniform over such a long period as that during which the patients in the present series were treated (1927–1970) The most common practice has been surgery combined with irradiation However, surgical procedures particularly in the early part of the period were often not sufficiently radical

DISCUSSION

The prognosis of mucosal malignant melanoma of the head and neck region is generally considered as very poor Combining previously reported cases with those in their own study, Holdcraft & Gallagher (1969) found that the five year survival rate of malignant melanomas of the nasal and paranasal sinus mucosa was 11 per cent (24 cases of a total of 226 cases) Only one patient (0 5%) survived for ten years In a series of 26 cases with malignant melanoma of the upper respiratory tract and oral cavity described by Moore & Martin (1955) only one patient survived for more than five years From the review of the reports up to 1969 Holdcraft and Gallagher found that the five year survival rate varied from 6 to 17% in cases with mucosal malignant melanoma in the nasal cavity and paranasal sinuses, which is the most comm[...] site for this tumour in the mucosal membra[...] of the head and neck

The five year survival rate in the pres[...] series was 17% (7 of the 41 cases) Thu[...] five year survival rate of mucosal malign[...] melanomas of the head and neck [...] ally is reported to be below 20% Even in [...] series described by Catlin (1967) and [...] man et al (1973) a higher incidence of five y[...] survival rate is found and a few cases w[...] clinical course prolonged over more than [...] years are reported, the long term prognosis [...] mucosal malignant tumours is extremely po[...] Thus, the ten year survival rate was in [...] present series 7% (3 of 41 cases) One of [...] three patients with a survival of over 10 ye[...] was alive and free of tumour symptoms at [...] last observation 16 years after the first sym[...] toms of the tumour, whereas the other t[...] patients in this group died of the [...] disease 15 and 35 years respectively after [...] first symptoms of the tumour The two [...] mentioned cases of mucosal malign[...] melanomas are unique as regards their p[...] longed clinical course despite regional lym[...] node metastases in an early stage of [...] disease and the repeated local recurren[...] Cutaneous malignant melanoma with this cl[...] ical course are, however, earlier [...] which can be explained by the fact that [...] a definite difference in the prognosis a[...] therefore also in the survival time betwe[...] patients with mucosal and cutaneo[...] malignant melanoma (Allen & Spitz 19[...] Moore & Martin, 1955, Catlin 1967, Enero[...] & Moberger 1973, Eneroth, 1975a and [...] The difference in prognosis between cutan[...] ous and mucosal malignant melanoma is a[...] plicable to all skin and mucosal membran[...] (respiratory alimentary and urogenital tract[...] The reason for the prognostic difference b[...] tween the mucosal and cutaneous maligna[...] melanomas of the head and neck region [...] discussed in the literature (Moore & Marti[...] 1955, Mason & Friedmann 1955 Cron[...] 1966) Delay in the treatment of the maligna[...]

lanomas in the mucosa of the upper respiratory tract is regarded as the most prominent son for the poor prognosis of these tu urs Whereas cutaneous malignant mela mas are usually clearly visible from an early te the mucosal malignant melanomas of head and neck region are frequently quies-t at the onset and produce insufficient nptoms to force the patient to seek medi advice in the early stages of the disease us when the condition at last is revealed, cal surgical removal of the tumour is im ssible Furhtermore, anatomical barriers pose extreme radical surgery in lesions of upper respiratory tract compared to cutaus lesions Therefore, an increased knowl of the initial symptoms of the mucosal gnant melanomas is the only means of ing an early diagnosis possible, and estial for radical surgyer of these tumours

Another reason for the difference in prog is between mucosal and cutaneous ignant melanoma can be a difference in the ological features Thus as compared to ineous malignant melanomas of the head neck (Eneroth & Moberger 1973) the ignant melanomas of the nasal cavity and inasal sinuses (Eneroth, 1975*a*) and the cavity (Eneroth 1975*b*) seemed to be e highly malignant histologically with great inaplasia, pleomorphism and higher incice of mitosis

n a comparison between primary cutaneous ignant melanoma and mucosal malignant lanoma of the head and neck region it could shown that the mucosal lesions occurred in her age groups than did the cutaneous le ns In the present study 80% of all patients re more than 50 years old at the first tological verification of the tumour Coming large series Allen & Spitz (1953) have own that cutaneous malignant melanomas curred only in 43% after the age of 50 years ereas mucosal malignant melanomas of the sal cavity and paranasal sinuses occurred in tients older than 50 years in 87% of the ses In the literature ... younger than 30 years with mucosal malignant melanomas only occasionally are described

Patients with mucosal malignant melanomas often have a very short survival time even though the primary lesion is small and the local surgery extensive Local recurrences as well as metastases to regional lymph nodes, however, do not preclude the ultimate survival in malignant melanoma in contrast to most other forms of malignant tumours This is elucidated by the two cases in the present study reported separately (case reports) The biologic behaviour of these two malignant melanomas is very curious Despite relatively limited primary excisions of the tumours, repeated local recurrences and regional lymph node metastases in an early stage of the disease, these two patients survived for 15 and 35 years, respectively, before they died in the tumour disease From these observations, the value of repeated surgical procedures of local recurrences and regional lymph node metastases is established Furthermore, a careful follow up of these tumours is stressed by the fact that even after long intervals with out symptoms (in one case 24 years) the tumour can recur This indicates a never-ceasing death risk in the malignant melanoma disease no matter how long after treatment the patient lives This probably represents quiescent disseminated disease held in check by a competent immunologic system (Freedmann et al , 1973)

ZUSAMMENFASSUNG

Eine erneut aufgenommene histologische Untersuchung und Klassifikation von primaren Schleimhauttumoren im Kopf und Halsbereich die in der Zeitspanne von 1927 bis 1970 in Radiumhemmet und Karolinska Sjukhuset in Stockholm behandelt worden waren erbrachte dass 41 Tumoren maligne Melanome waren Alle diese 41 Tumoren waren auf die Nasenhaupthöhle die Nasen nebenhohlen und die Mundhohle begrenzt An andern Stellen des Kopf und Halsbereichs wurde kein einziger Fall von primaren malignen Schleimhautmelanomen gefunden

In der vorliegenden Arbeit ist die Langzeitprognose analysiert worden Die Beobachtungsperiode war im

auf 48 Jahre Das Ergebnis war, dass maligne Schleimhautmelanome eine sehr schlechte Prognose hatten, nämlich eine Überlebensrate von 17% für den Zeitraum von fünf Jahren (d h bei sieben von insgesamt 41 Fällen) und von 7% für den Zeitraum von zehn Jahren (bei drei von insgesamt 41 Fällen) Die Unmöglichkeit einer Voraussage des klinischen Verhaltens dieses Tumortyps veranschaulichen die Falle mit verlangertem klinischen Verlauf trotz einer primär relativ begrenzten operativen Behandlung mit wiederholten Lokalrezidiven und regionalen Lymphknotenmetastasen in einem fruhen Stadium der Erkrankung

Es besteht daher bei diesen Tumoren immer das standige Risiko eines todlichen Ausgangs, wenn einmal ein malignes Melanom aufgetreten ist Aus diesem Grunde ist eine äusserst sorgfältige und lebenslange Beobachtung von Tumorpatienten und der Wert wiederholter operativer Behandlung bei Lokalrezidiven und regionalen Lymphknotenmetastasen besonders hervorzuheben

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C-M Eneroth M.D
Dept of Otolaryngology
Karolinska Sjukhuset
S-10401 Stockholm
Sweden

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INCREASED LYMPHOCYTE ATP-ASE ACTIVITY IN PATIENTS WITH CARCINOMAS OF THE ORAL CAVITY AND LARYNX

J Ellegaard, H Traunberg, B Dorff and V Esmann

From the Department of Medicine and Epidemiology Marselisborg Hospital Aarhus Denmark

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'act Increased mitochondrial ATP-ase activity was l in circulating lymphocytes from 5 out of 8 patients carcinoma of the oral cavity or oropharynx and from t of 10 patients with carcinoma of the larynx In a nty of these cases changes in the ATP ase activity treatment paralleled to the clinical result of treat It is suggested that determination of ATP ase activ f circulating lymphocytes is of diagnostic value in nts with oral oropharyngeal and laryngeal car nas as well as of prognostic value after treatment of atients

dence has accumulated indicating that the ıll thymus dependent lymphocytes (the ells) are the main effector cells in the host ence against growth of neoplastic cells u et al , 1967, Bubenik et al , 1970, DiSaia l , 1971, Richters et al , 1971, Hellström et 1971*a* Perlmann et al , 1972), although the mus non-dependent lymphocytes (the ells) also can participate in the target cell ıtruction (Henney et al , 1972, Perlmann et 1972) The killing takes place when the nphocytes, presumably sensitized by cir-ating tumour-associated antigens obtain ıse contact with the malignant cells (Humble al , 1956, Wilson, 1963, Ax et al , 1968, chters & Sherwin, 1974) It is not yet fully derstood how the lymphocytes destroy the cancer cells, but the process is thought in itiated by activation of a membrane-bound ATP ase followed by a series of intracellular biochemical reactions ending with a nuclear activation and transformation of the lympho-cytes The energy required for these reactions may be provided by hydrolysis of ATP and in previous experiments we have demonstrated the presence of an increased ATP ase activity in circulating small lymphocytes isolated from patients with malignant tumours (Ellegaard & Dimitrov, 1972*a*)

The ATP ase activity present in the supernatant of homogenized lymphocytes was found to be stimulated by Mg^{2+} and dinitro-phenol and inhibited by oligomycin, but re latively independent of Na^{+} and K^{+} and in-sensitive to ouabain This serves to characterize the enzyme as a mitochondrial ATP ase and in a few experiments it was dem-onstrated that this enzyme activity is actu ally confined to the mitochondrial fraction of the cytoplasma and constitute a greater part of the ATP hydrolyzing capacity of the super natant of a crude lymphocyte homogenate (Ellegaard & Dimitrov, 1972*a*)

The present report demonstrates that the ATP ase activity of lymphocytes is increased in patients with carcinomas of the oral cavity and larynx and that this activity decreases upon successful treatment

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Table I. *Clinical data and lymphocyte ATP-ase activity before and 6–12 weeks after treatm in 8 patients with oral and oropharyngeal carcinomas*

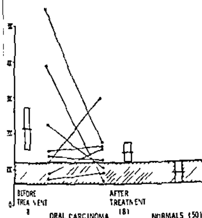
Initials	Age	Sex	Localisation of tumour	Tumour stage	Effect of treatment	ATP ase before	ATP ase after
T N	69	M	Oropharyngeal	$T_1N_0M_0$	Complete	222	104
J A P	74	M	Oral bottom	$T_2N_0M_0$	Complete	73	90
P L	70	F	Gingival	$T_2N_1M_0$	Incomplete	134	128
L O P	70	M	Tongue	$T_3N_0M_0$	Complete	543	174
O P O	85	M	Buccal	$T_3N_1M_0$	Incomplete	90	165
L T	62	M	Buccal	$T_3N_3M_0$	Incomplete	152	165
E M L	57	M	Oropharyngeal	$T_3N_3M_0$	Incomplete	119	300
K J	50	M	Oral bottom	$T_4N_3M_0$	Complete	389	66

MATERIAL AND METHODS

The study included a control group of 50 normal healthy subjects of both sexes (age 22–88 years), a group of 10 patients (age 47–83 years) with larynx carcinomas, and a group of 8 patients (age 50–85 years) with oral or oropharyngeal carcinomas All the cancer patients had proven histological diagnoses and were staged according to the UICC classification (Tables I and II) Before therapy none of the carcinomas showed distant metastases and were thus all staged M_0 One of the larynx carcinomas (T_3) had spread to the regional lymph nodes (N_1), while the remaining were small localised tumours staged T_1 in 5 cases T_2 in 4 cases Of the oral and oro yngeal tumours 3 were localised while 5 wed regional lymph node metastases staged N_1 in 2 cases and N_3 in 3 cases The tumours were staged T_1 in one case, T_2 in 2 cases, and T_3–T_4 in the remaining cases The lymphocyte ATP-ase activity was determined shortly before and again 6–12 weeks after treatment, in one case however, 30 weeks after treatment In all cases therapy was given as high-voltage irradiation (6000 rad) In one case (K J), a hemimandibulectomy with resection of the regional lymph nodes was also performed The result of irradiation therapy is given as complete when the primary tumour no longer was visible or palpable and no signs of metastases had developed upon follow-up 6–12 weeks later

The lymphocytes were isolated as previously described (Ellegaard & Dimitrov 1972*a*) from 50 ml heparinized blood In ord to remove the most sticky population platelets, the blood was passed slowly throu a 5 cm column of washed 0 1 mm glass bea in a 10 ml plastic syringe This prevents di turbing platelet aggregations during the sub sequent isolation of the lymphocytes wi the Ficoll-technique of Boyum (1968) Th harvested lymphocytes were washed in salin and residual platelets and red cells removed b suspending the cells in 5 ml 10% sucrose an centrifuging for 15 min at 150 *g* on a discor tinuous sucrose gradient made up of 25 n 16% sucrose and 4 ml 20% sucrose In rar cases a final 5 min exposure to a 1% am monium oxalate solution was necessary to ly a few remaining red cells

The intact lymphocytes were now counted suspended in cold 0 25 M sucrose (10^6 cells pe ml) and sonicated for 2×15 sec under cool ng The resulting homogenate was spun at 1000 for 10 min in a refrigerated centrifuge and th supernatant used for the ATP ase assay (El legaard & Dimitrov, 1972*a*) In this the libera tion of inorganic phosphate (P_i) from the sub strate, Tris-ATP, during a one hour incubation at 37°C is determined The results were cor rected for endogenous P_i and non-enzymatic P_i-release during the incubation Double incu bations and double determinations on each in cubation were used throughout The final re



l Lymphocyte ATP ase activity in 8 patients with and oropharyngeal carcinomas before and 6–12 weeks r treatment compared to the ATP ase activity in 50 lhy controls *The columns express the group mean* EM and the shaded area the normal mean ±1 S D

s are expressed as nmoles P_i liberated per r per mg protein of supernatant, the pro- concentration being determined according owry et al (1951)

ll reagents were of analytical grade Ficoll purchased from Pharmacia, Sweden and -ATP from Sigma, USA

RESULTS

l and oropharyngeal carcinomas

: staging of the tumours and clinical re- nse to treatment of 8 patients with oral and pharyngeal carcinomas are presented in ble I Also shown are the lymphocyte ATP-ase activities before and after treatment The histological diagnoses were adeno- carcinoma in two cases and squamous cell carcinoma in 6 cases The ATP ase activity before treatment averaged 215±166 (S D) nmoles P_i/mg protein/hour, which is significantly higher ($p<0\ 02$) than the activity in lymphocytes from 50 normal controls (91±33) (cf Fig 1) After radiation therapy the ATP-ase activity decreased to an average of 149±72 (S D)

Table I shows that in the four patients where the effect of treatment was deemed complete, the ATP ase activity decreased dramatically in three, while the activity in the fourth patient remained unchanged within the indicated range Among the four patients with an in complete effect of treatment, the ATP-ase activity increased greatly in two cases and slightly in one case This paralleled to a deterioration of the clinical condition and all these patients died within a year The ATP-ase activity of the fourth of these patients remained unchanged at follow up (134 vs 128) and it is known that this patient was still alive one year after the investigation was com pleted

Laryngeal carcinomas

Table II shows the staging of the tumours and the clinical response to treatment in 10 patients with squamous cell carcinomas of the larynx as well as the ATP ase activities before

ble II *Clinical data and lymphocyte ATP ase activity before and 6–12 weeks after treatment 10 patients with laryngeal carcinomas*

uals	Age	Sex	Localisation of tumour	Tumour stage	Effect of treatment	ATP ase before	ATP ase after
B K	50	M	Supraglottical	$T_1N_0M_0$	Complete	167	123
M K	68	M	Supraglottical	$T_3N_0M_0$	Incomplete	158	84
D	58	M	Vocal cord	$T_1N_0M_0$	Complete	79	177
K N	83	M	Vocal cord	$T_1N_0M_0$	Complete	66	115
C	65	M	Vocal cord	$T_1N_0M_0$	Complete	233	97
M N	47	M	Vocal cord	$T\ N_0M_0$	Complete	224	104
P	69	M	Infraglottical	$T_2N_0M_0$	Complete	68	103
R	72	M	Infraglottical	$T_2N\ M_0$	Complete	103	83
V K	80	M	Infraglottical	$T_1N\ M_0$	Incomplete	117	200
A M	65	M	Infraglottical	T_1N_0M	Complete	343	110

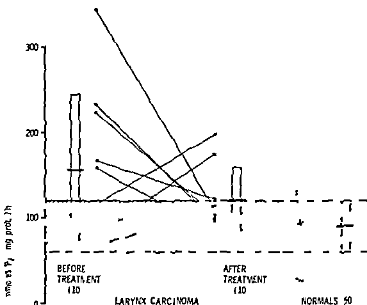


Fig 2 Lymphocyte ATP-ase acti y in patients with laryngeal carcinomas bef and 6-12 weeks after treatment com to the ATP-ase act vity n 50 healthy trols The columns express the group me +S E M and the shaded area the norm mean +1 S D

and after treatment The average ATP ase activity before irradiation was 156±89 (S D) which is significantly higher ($p<0\ 02$) than that of the controls

In 8 patients the effect of treatment was evaluated as complete upon the immediate follow up In four of these cases (GBK AC TMN & CAM) the ATP ase activity fell from high values to within the indicated range and in one case (AR) the activity fell slightly within the indicated range (Fig 2) In the remaining ... patients (VD KKN & VP) the ATP ase ...vity was definitely increased at follow up In one of these three cases (VP) a clinical relapse was later established while in the other two (KKN & VD) no sign of relapse has been noticed so far

In 2 patients the effect of the treatment was evaluated as clinically incomplete In one case the ATP ase activity increased and in the other case the activity had decreased upon follow up 12 weeks later

DISCUSSION

The reason for the elevated activity of lymphocyte mitochondrial ATP ase in patients with carcinomas is not know Stimulation of the lymphocyte plasmamembrane by tumo... antigens (Jehn et al 1970) and phytohaema... glutinin A (PHA) (Craddock et al 1971) re... sults in increased protein RNA and DN... synthesis and in blastic transformation of the cells Stimulation by PHA leads to increa... synthesis of phosphoproteins and to decrease intracellular concentration of ATP (Kle... smith et al 1966) as well as an increased ac... tivity of the mitochondrial ATP-ase (Ellegaar... & Dimitrov 1972*a*) We suggest that s... reactions might take place *in vivo* upon stimu... lation of the lymphocytes by circulating tumour associated antigens

Measuring the lymphocyte ATP ase activity might be of value as a diagnostic aid in cancer Previously only a few other biochemical tests for cancer have been developed and the most widely used of these has been the demonstra... tion of increased serum concentrations of the carcinoembryonic antigen (Dykes & King 1972) This test is however almost invali... dated by a high rate of falsely positive results (Martin et al 1972) Aside from neoplastic diseases the lymphocyte ATP ase activity has only been found significantly elevated in pa... tients with ulcerative colitis and hepatic amoebiasis

n the present series of patients with oral, pharyngeal or laryngeal carcinomas, the ase ATP ase activity was significantly ated compared to normal From inspec- of Figs 1 and 2, it is, however, evident a normal pretreatment value of ATP ase not exclude the presence of these gnancies For example, in one patient with al carcinoma and lymph node involve- the ATP ase activity was low before ment and did not increase after therapy te the result of treatment was recorded as ally incomplete A possible explanation uch a result might be sought in the fact lymphocytes isolated from a malignant ur or its regional lymph nodes in many show anergy against the tumour cells, ibly due to immunological paralysis d by blocking antibodies (Hellstrom et 971*b*, Nind et al , 1973)

s 1 and 2 also show that values above dicated range (mean ±S D) naturally are in normal subjects However, ATP-ase ties of more than 2×S D above the nor- iean value would be highly suggestive of esence of malignancies and such values found in one third of the patients with r oropharyngeal carcinomas and in more one half of the patients with laryngeal iomas, which under the circumstances the ATP ase test a reasonable sensitive ieter

on follow up 6–12 weeks after treatment, ges in ATP-ase activity in lymphocytes patients with oral or oropharyngeal car- ias corresponded to improvement or de- ation of the clinical condition and a pro- ced rise in ATP-ase activity bore a grave iostic sign In case of the laryngeal nomas, the results at follow up were not iambigous In three of eight cases, where linical evaluation showed that the treat- had been effective the ATP-ase activity rtheless increased significantly In one of ases, however the clinical evaluation was ig as a relapse was found on a later w up Whether the same result will be- come evident for the two remaining patients, and the biochemical changes thus shown to precede the clinical evaluation of relapse of these low grade tumours, remains to be seen

The ATP ase activity of circulating lymphocytes has previously been found elevated in patients with carcinoma of the lung (Ellegaard & Dimitrov, 1972*b*), gastrointestinal tract (Dimitrov & Ellegaard, 1972*b*), uterine cervix (Ellegaard et al , 1974*b*) and the urinary bladder (Ellegaard et al , 1974*a*) The test also seems to be of diagnostic value in cases of carcinomas of the oral cavity and larynx and of prognostic value after treatment

ACKNOWLEDGEMENTS

We are indebted to Mrs Kate Sommer for expert technical assistance and we also want to thank our colleagues at the Department of Oto-rhino-laryngology Aarhus Kommunehospital and at the Radium Station Aarhus for kind cooperation

ZUSAMMENFASSUNG

Erhohte mitochondriale ATP-ase Aktivitat wurde in den Lymphozyten im Kreislauf bei fünf von acht Patienten mit Karzinom der Mundhöhle oder Oropharynx und bei funf von sehn Patienten mit Carcinoma laryngis ge funden Bei den meisten Fallen wurden Veranderungen in der ATP ase Aktivitat nach der Behandlung parallel zum klinischen Ergebnis der Behandlung gefunden Es wird angenommen dass die Bewertung der ATP-ase Aktivitat der zirkulierenden Lymphozyten sowohl von diagnostischem Wert bei Patienten mit oralen oro- haryngealen und laryngealen Karzinomen als auch von prognostischem Wert nach der Behandlung ist

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DK-8000 Aarhus C
Denmark

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THYROPLASTY TYPE I (LATERAL COMPRESSION) FOR DYSPHONIA DUE TO VOCAL CORD PARALYSIS OR ATROPHY

N Isshiki, H Okamura and T Ishikawa

From the Department of Otolaryngology School of Medicine Kyoto University Kyoto Japan

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ct Based on the experimental results of thyroplas /roplasty type I which aims at medical shifting the :ord was performed on 8 patients with dysphonia 6 ocal cord paralysis and 2 with vocal cord atrophy ırgery was conducted on either in or out patient ınd local anesthesia was used Usually a rectangu ısion was made on the thyroid cartilage at the level vocal cord and the fragment was depressed inward ilage pie e taken from the opposite side was used as lge if necessary to enhance the effect of lateral 'ession of the vocal cord The voice after surgery ›nerally satisfactory except in one case of traumatic cord paralysis Complications such as stridor or ea were nil As surgical intervention inside the d cartilage is minimal fine and reliable adjustment ›ression is possible during the surgery

previous paper (Isshiki et al 1974) we rted experimental results of thyroplasties h aim at modifying the position and ten of the vocal cords The experimental y on 14 dogs indicated the usefulness of ›us types of reformation of the thyroid lage for dysphonias artificially produced the procedures such as sectioning the rrent laryngeal nerve From the functional ' points 4 types of thyroplasty were pro :d 1 lateral compression 2 lateral ex ııon 3 relaxation (shortening) and 4 :ching (lengthening) of the vocal cord The ent paper describes the surgical technique results of thyroplasty type I (lateral com ısion) This surgery aims at shifting the al cord medially through deformation of the thyroid cartilage and was performed on 8 patients with dysphonia 6 with unilateral vocal cord paralysis and 2 with vocal cord atrophy

INDICATIONS

Any organic laryngeal disease which caused imperfect closure of the glottis during phonation without swelling or tumour of the vocal cord is an indication for thyroplasty type I Most frequently it is indicated for dysphonia due to unilateral vocal cord paralysis Thorough physical examination for detecting possible underlying disease is a prerequisite to any treatment of vocal cord paralysis Furthermore the patient should be followed up either on voice training and/or medication for at least 6 months after the onset of the disease in order to determine the underlying disease as well as the position and immobility of the involved cord Oval glottal chink on phonation due to vocal cord atrophy or other diseases may be an indication for thyroplasty especially when the chink is large Functional aspects or daily fluctuation of laryngeal findings and the degree of hoarseness should be taken into account prior to the decision of performing thyroplasty

Manual test of the larynx is most useful for predicting the outcome Manual compres

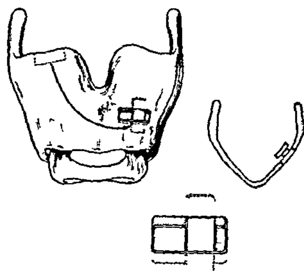


Fig 1 The most typical thyroplasty type I The rectangular incision 10 mm×3 mm the upper margin of which is at the level of the vocal cord A wedge may be inserted between the rectangular fragment and the remaining frame of the thyroid cartilage to enhance the compression effect when necessary

of the bilateral thyroid alae at various points and/or bimanual approximation of the cricothyroid distance gives us a fairly good indication as to where and to what extent the thyroid cartilage needs to be deformed to obtain a maximum improvement in voice Other preoperative tests include voice recording tomography and measurement of air flow rate during phonation Laryngoscopic photographs are also taken when necessary In cases of slight hoarseness with the vocal cord fixed in a paramedian position thyroplasty is not the surgery of choice

The other factors to be taken into consideration in deciding the indication of thyroplasty are 1 laryngeal findings 2 type of the proceeding surgery and 3 age A scar or granulation on the vocal cord is associated with poor prognosis (Case 3) The thyroid cartilage may have sustained little or much damage after surgical intervention on the thyroid gland (Case 6) Surgery on the thyroid cartilage of the aged is generally difficult to perform due to advanced calcification and may require use of an oscillating saw (Case 11)

SURGICAL PROCEDURES

The surgery can be performed on both in and out patients Two of our patients were ambulatory while the other six were hospitalized for 2–3 days The surgery is performed under local anesthesia with 1% Xylocaine The patient is in the supine position with the head extended Incision is made horizontally on the anterior neck at the level of the midpoint between the thyroid prominence and lower margin of the thyroid cartilage The thyroid cartilage is well exposed by separation of the bilateral sternohyoid muscles and additional Xylocaine is injected into the thyroid perichondrium

The types of incisions in the thyroid ala var with the cases The most typical incision no being used is a rectangular one 10 mm×3 mm the upper margin of which should be at the level of the vocal cord (Fig 1) Prior t incision the rectangular window is marke with pyocutanine so that the upper horizont line can be located at the midpoint between th thyroid notch and lower margin of the thyroi cartilage and can be extended further back parallel to the lower margin of the thyroid al If the fragment to be depressed is located to high it may cause bulging of the false voca cord or Morgagni s ventricle although w have never experienced such an occurrence An anatomical study on the human cadave larynx indicated that the anterior commissu of the vocal cords (upper surface) is position almost at the level of the midpoint between th thyroid notch and lower margin of the thyro cartilage or slightly higher than that level Details of the measures will be given else where Incision is usually done using a No 11 BP blade but may require an oscillating saw (narrowest gingko-shaped blade) in case of ossified cartilage (Case 11) Too deep an incision and elevation beyond the inner peri chondrium should be carefully avoided in order to minimize swelling or edema of the vocal cord After gentle and careful marginal separation of the rectangular fragment with a rather fine raspatrium the fragment is de

ssed inward to varied depths to determine at degree of depression is optimal for voice oduction

During this procedure of adjusting depression to the best obtainable voice, the extended d should be repositioned straight to avoid judgement due to the abnormal head position If only slight inner displacement of the ment is optimal for voice production, the ressed rectangular fragment is shifted 1 downward (caudal direction) behind the aining frame of the thyroid alae If further er displacement is required for better voice duction a wedge should be inserted between the rectangular fragment and the re ning frame of the thyroid Usually a wedge aken from the upper margin of the opposite roid ala and may be pared to an adequate kness The wedge is usually fixed with the roid ala by one or two 4 0 nylon through through sutures Varieties of incisions h as two parallel incisions have also been d, as shown in Figs 1–6, 8, 9 At this stage clinical experience, no conclusion can be wn as to which is the best incision to be d but the rectangular incision with or hout a wedge appears most effective with imal intervention The optimal type inci n still remains as a future problem for dy

In cases where the paralysed cord is situ d higher than the intact one cricothyroid proximation should be tested to determine ether there is further improvement in the ce It is usually done by pulling a 3-0 nylon ure between the thyroid and cricoid carti es If voice improvement is obtained by the itative pull, the thyroid and cricoid carti ges should be fixed in approximation with e or two 3-0 nylon mattress sutures For evention of perichondritis antibiotic powr is usually applied to the field before osure of the wound The bilateral sternoroid is sutured together to cover the carti ge For cosmetic reasons closure of the skin cision should be done most carefully Slight edema of the involved vocal cord may last for several post-operative days but never to the extent of causing stridor or dyspnea In one case (No 11) dark red discoloration of the false vocal cord suggestive of submucous hematoma was noted, on the side from where the wedge had been taken This reddishness disappeared in a week

RESULTS

Voice improvement was obtained in all operated patients, although the result in case of traffic trauma was unsatisfactory Cases of thyroplasty I so far performed are too few to assess the results statistically Therefore, each case will be described chronologically The results were evaluated through auditory evaluation based on semantic differential methods, sonagram, computer analysis of pitch perturbation (mean of the cycle-by-cycle variation of the fundamental frequency expressed in semi tone=mean Δf in semi tone) (Table I), and measurement of air flow during phonation

Case No 2 S M , a male aged 27, with left vocal cord paralysis, which occurred after surgical removal of a jugular glomus tumour on the left side of the neck 1 year and 9 months

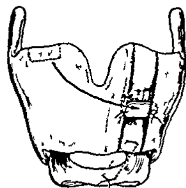
Table I *Pitch perturbation in semi tone*

Case No	Sex	Before surgery	After surgery
2	m	Unmeasurable	0 31
5	m	1 30	0 18
6	f	0 56	0 18
9	m	1 43	0 17
11	m	1 20	0 28
4	f	0 26	0 17
8	f	0 41	0 20

Pitch perturbation was expressed as the mean of the cycle by-cycle variation of the fundamental frequency in semi tone

$$\overline{\Delta f} = \frac{\sum \left| F_i - F_i \right|}{N-1}$$

where F is an instantaneous frequency in semi tone and N is a number of measured waves The mean value range of 95% confidence limit for the normal 0 16 and 0 08 ~ 0 23 for male and 0 19 and 0 ... female respectively



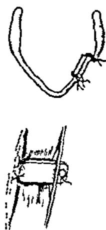


Fig 2 Thyroplasty type I and IV for case No 2

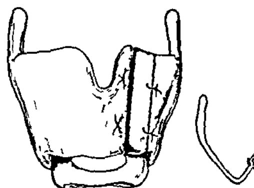


Fig 3 Thyroplasty type I for case No 3 The pos one third portion of the thyroid cartilage was attemp slip in beneath the middle one third to increas compression effect at the posterior glottis The l portion however, was not markedly displaced med presumably because the cricoid cartilage blocke medial displacement of the lateral one third thyroid a

previous to the surgery The surgical record indicated that the vagal nerve had been sectioned at the time of surgery His voice was extremely breathy and weak Indirect laryngoscopy revealed the left vocal cord fixed almost in a lateral position The fixed cord was found on radiotomographic examination to be positioned much higher than the intact cord

Thyroplasty I and IV was performed as illustrated in Fig 2 Two parallel vertical incisions were made on the left ala and the midportion of the thyroid ala was depressed inwards The improvement of voice was insufficient A cartilage segment was removed from the upper margin of the opposite ala and used as a wedge to reinforce the compression effect particularly at the posterior part of the vocal cord Since combined paralysis of the recurrent and superior laryngeal nerves was evident in this case, cricothyroid approximation was added to the above procedure, resulting in further improvement of voice Although the postoperative improvement of voice was remarkable, the voice was still slightly hoarse and a minute difference in level between the two vocal cords was still evident on tomogram

Case No 3 K Y, a male aged 21 left vocal cord paralysis after traffic trauma to the neck The patient had been severely hoarse for 7 years after the accident Indirect laryngoscopy revealed the left vocal cord fixed in an termediate position and bilateral scar for tion particularly in the subglottic region phonation, a triangular chink was noted at posterior glottis Manual inward depressio the superior horn of the thyroid cartil produced some improvement of voice Thy plasty I was performed as illustrated in Fig

First, a vertical incision was made on left thyroid ala at anterior-middle third and lateral part was slipped in beneath the me part The voice improvement was not rema able On the basis of the clinical finding t the glottal chink was wide posteriorly, anot vertical incision was made and the poster one-third portion was slipped in beneath t middle one third to increase the compressi effect at the posterior glottis However, t lateral portion was not markedly displac medially, presumably because the cric cartilage blocked the medial displacement the lateral one third thyroid ala Postoperati improvement of voice was not to the degr expected before surgery though the glott chink during phonation was much reduced laryngoscopy It was assumed that the sc tissue on the vocal cord may be greatly r sponsible for the rather unsatisfactory result

Case No 5 T T, a male aged 36, left voc

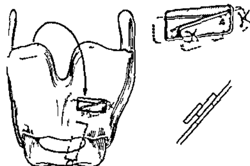


Fig 4 Thyroplasty type I and IV for case No 5 A wedge was fixed with two 4-0 nylone sutures

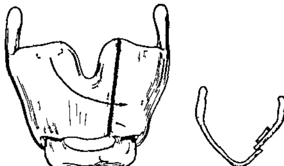


Fig 5 A typical thyroplasty type I and IV for case No 6 The cartilage fragment was inserted beneath the thyroid ala Due to the fragile quality of the thyroid ala typical thyroplasty type I was impossible

cord paralysis attributed to pulmonary tuberculosis with a 6 month history of hoarseness The left vocal cord was fixed in an intermediate position and positioned higher than the healthy one on tomogram Manual compression of the thyroid alae inwards and cricothyroid approximation resulted in an almost normal voice

The typical rectangular incision was made on the left thyroid ala as shown in Fig 4 A cartilage fragment was taken from the opposite side pared off to a half thickness and fixed as a wedge inside the rectangular window Cricothyroid approximation further improved the voice The thyroid cartilage was partly calcified but not to the extent to make incision with the scalpel difficult The voice after surgery was almost normal as shown in Table I

Case No 6 K O a female aged 48 left vocal cord paralysis after surgery on the thyroid gland Her voice had been hoarse and weak postoperatively Exposure and vertical incision on the thyroid cartilage as shown in Fig 5 revealed fragility particularly at the median and lower part of the thyroid cartilage which was seemingly ascribed to electrocoagulation during the preceding surgery The lateral fragment was slipped in beneath the median portion as was done in cases 2 and 3 but due to the fragile nature of the median portion of the thyroid cartilage the inward compression effect was insufficient To enhance inward compression a cartilage fragment taken from the opposite side was inserted beneath the left thyroid ala at the level of the vocal cord (Fig 5) The lateral part of the left ala was again slipped in beneath the median part

The left cricothyroid muscle was scarred and atrophic and did not contract on phonation in contrast to the right cricothyroid muscle A 3 0 nylon suture was tied to shorten the cricothyroid distance Again due to the fragility a strong pull was impossible The voice after surgery was close to normal but on occasion slightly fluttering in quality especially when the head was inclined toward the right The difference in level between the two cords was not completely corrected

Case No 9 K T a male aged 27 right vocal cord paralysis of unknown etiology (intermediate position) The manipulation test indicated good prognosis after surgery Typical rectangular form thyroplasty I with a wedge was performed (Fig 6) Cricothyroid approximation was not done in this case because the tentative cricothyroid approximation resulted in a rather cracking voice The voice after surgery was quite satisfactory as shown in Fig 7

Case No 11 S N a male aged 64 left vocal cord paralysis of unknown etiology of 1 year 7 months duration The vocal co
fixed in an intermediate position
difference from the intact cord

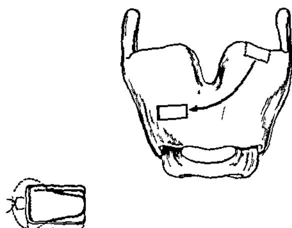


Fig 6 Thyroplasty type I for case No 9 A wedge is used to increase the lateral compression particularly at the posterior portion of the vocal cord

tion test indicated good prognosis The rectangular incision was made in the thyroid ala, which was so calcified that an oscillating saw was required for cutting

The narrowest gingko shaped blade was used to cut 80% through the full thickness No 11 blade was used to complete the in sion An almost normal voice was obtair when the rectangular fragment was depres deep inside A wedge was taken as usual an half thickness wedge was inserted perpendi lar to the rectangular window (Fig 1) T procedure of inserting the wedge was ve difficult and required considerable care Po operatively, the right false vocal cord w reddish and swollen for a week probably d to slight hematoma after removal of the car lage fragment As the swelling subsided t voice improved almost to a normal quality

Case No 4 A I , a female aged 30 voc cord atrophy Three years previously she h used oral contraceptive pills for a few mont Her voice had been hoarse since then Indire laryngoscopy revealed a marked oval glott

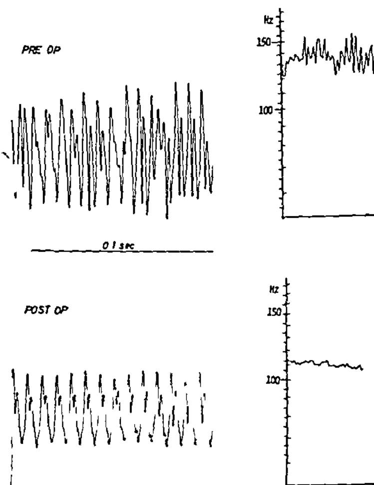


Fig 7 The acoustic wave of voice (left) and pitch perturbation (right) in case No before and after surgery For pitch pertu bation graph the ordinate indicates f quency scale in semi tone while the a scissa indicates the consecutive number sound waves The pitch perturbation w markedly reduced after surgery

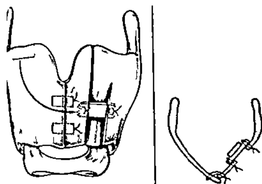


Fig 8 Thyroplasty type I for case No 4

nk during phonation despite apparently rmal movement of the bilateral vocal cords ither scar nor sulcus was noted on the vocal ds Voice training did not result in im vement in the glottal condition but the lth of the glottal chink changed day by day ied on the finding that her voice was grealy proved by manual compression of the roid cartilage she was hospitalized for roplasty type I The thyroid cartilage was ised vertically in the median line and the ala was shifted inward and fixed with 4-0 on sutures (Fig 8) The voice was much proved during surgery

The laryngoscopic findings on the next day re rather discouraging with a noticeable nk still remaining on phonation particu y at the posterior glottis Her voice re ssed after surgery The possible factors re onsible for the change of voice from the time surgery may be 1 extended neck at the ie of surgery 2 postoperative laryngeal in which may suppress a normal contraction the laryngeal muscles during phonation 3 stoperative edema

After being followed up for 3 weeks she is reoperated to reduce the posterior glottal ink during phonation Two vertical incisions ere made on the left thyroid ala as shown in g 8 The middle cartilagenous portion with e pedicle upward was depressed inward with wedge taken from the opposite side ala The edge was fixed with two 4-0 nylon sutures fter this second surgery her speaking voice was much improved but the glottal chink noted on laryngoscopy during phonation still remained though to a lesser degree The degree of imperfect closure of the glottis fluctuated greatly day by day

Case No 8 K T, a female aged 18, vocal cord atrophy She had a complaint of laryngeal pain on phonation, slight hoarseness, weak and too-high pitched voice of 8 years duration Voice therapy in an attempt to lower the pitch was ineffective Linear chink during phonation was noted on indirect laryngoscopy Slight compression of the thyroid ala improved the pitch and quality of voice Typical thyroplasty type I without a wedge was performed (Fig 9) According to her statement, she became com pletely free from pain on phonation one week after surgery Her voice was very much im proved in quality but still sounded slightly asthenic

DISCUSSION

Our previous report covered the various surgi cal methods developed by many surgeons for correcting the fixed vocal cord position Among them teflon or silicon injection or cartilage implantation are presently the most popular Intrachordal injection of plastic ma terial is certainly a good and simple method to bring the vocal cord margin toward the midline However Bernstein & Holt (1967) pointed out possible disadvantages of the method such as neoplastic response or

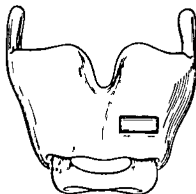


Fig 9 Thyroplasty type I for case No 8

eventual rejection by the tissue of the synthetic materials, migration or absorption of the implanted tissue, local stiffening of the vocal cord due to injection etc We also obtained excellent results in voice production by intrachordal injection of elastic silicon in many patients with vocal cord paralysis In a few cases, however, the results were unsatisfactory especially when too much elastic silicon was injected near the margin of the vocal cord in a second or third injection, resulting in rather stiff vocal cord In other words, one of the disadvantages of the injection method is that the volume and site of injection cannot be altered according to the voice, since the injected materials cannot be removed

The advantages of our methods of thyroplasty are 1 Adjustment of the degree of lateral compression is possible during surgery in accordance to the voice obtainable 2 Intervention inside the inner perichondrium of the thyroid cartilage is minimal Although our experiences with thyroplasty are not so extensive, we have encountered problems, as described for each case They were 1 extended head position 2 previous scar on the vocal cord or the thyroid cartilage, 3 calcified cartilage, 4 postoperative edema, 5 functional aspect of vocal cord atrophy

We have not yet concluded which type of incision on the thyroid ala is best After the experience with two vertical parallel incisions, we are inclined to prefer the rectangular type as it seems sufficiently effective and involves less surgical trauma This type of incision is very similar to that used by Payr (1915), but differs in so far as ours has no pedicle so that the fragment can shift quite easily toward the midline The site and size of cartilage incision should be based upon anatomical data of the human larynx Our data regarding various measurements of the human larynx which should be referred to when considering thyroplasty, will be reported elsewhere

In each case we examined whether or not the cricothyroid approximation by suture improves the voice after thyroplasty type I As i[illegible] well known, there is the possibility of c[illegible]bined recurrent and superior laryngeal nerve paralysis when the vocal cord is fixed in an intermediate position, according to Wagner[4] Grossman Our preliminary experimental study on unilateral paralysis of the cricothyroid muscle demonstrated that this paralysis alone does not cause hoarseness but rather there is a lag in the vibratory phase between the two cords If it is accompanied by imperfect closure of the glottis the results appear rather complex Such is now under study utilizing an extirpated larynx and a high speed motion camera The effect of cricothyroid approximation on the level of the vocal cord remains to be further investigated

In our experiments of sectioning the recurrent laryngeal nerve, additional sectioning of the ipsilateral external branch of the superior laryngeal nerve resulted without exception in relative elevation of the immobilized vocal cord by 1 mm and cricothyroid approximation by suture corrected that level difference of the two vocal cords On the basis of this experimental finding we make a tentative cricothyroid approximation a routine procedure during surgery in cases where the vocal cords are positioned at different levels Factors other than the cricothyroid muscle apparently affect the level of the vocal cord the more lateral the fixed position the higher is the one paralysed cord

Manipulation of the laryngeal cartilage before surgery not only indicates the post-op prognosis but also helps relieve the patient's anxiety as to what his voice would be like after surgery Our clinical experiences with thyroplasty I indicate its usefulness for dysphonia due to vocal cord paralysis or atrophy As experience accumulates modification of techniques will necessarily follow and these shall be reported in following papers

ZUSAMMENFASSUNG

Auf Grund der experimentellen Resultate der Thyreoplastik wurde Thyreoplastik Typ I über med ale Verschiebung

der Stimmbänder) bei acht Patienten mit Dysphonie (sechs mit Stimmbandlähmung, zwei mit Stimmbandatrophie) ausgeführt. Die chirurgischen Eingriffe wurden bei den entweder ambulant oder stationär aufgenommenen Patienten unter lokaler Anästhesie durchgeführt. Der rektangulare Einschnitt des Schildknorpels wurde im Seitenteil in der Höhe der Stimmbänder vorgenommen und das Bruchstück wurde nach innen gedrückt. Das von der anderen Seite herausgenommende Knorpelstück wurde wenn notwendig als Keil benutzt und die Wirkung der lateralen Expansion auf die Stimmbänder zu erhöhen. Nach dem Eingriff war die Stimme im allgemeinen genügend, ein Fall mit Stimmbandlähmung nach einem Verkehrsunfall ausgenommen. Es gab insgesamt keine Nebenwirkungen (Stridor oder Dysphoe). Weil der chirurgische Eingriff im Inneren des Schildknorpels minimal ist, kann die feine und sichere Regulierung des Niederdrucks intraoperativ ohne weiteres durchgeführt werden.

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N Isshiki M D
Dept of Otolaryngology
School of Medicine
Kyoto University
Sakyo ku
Kyoto 606
Japan

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IMMUNOLOGICAL FUNCTION OF HUMAN TONSIL

Surface Topology of Human Tonsil Lymphocytes using the Scanning Electron Microscope (SEM)

T Tabata, T Katsurahara, T Enomoto, N Kitashoji and S Tanaka

From the Research Institute for Medical Sciences, Wakayama Medical College Wakayama Japan

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Abstract The surface topology of the lymphocytes in human tonsils was performed by means of the scanning electron microscope (SEM) It was revealed that human tonsils consist of both 40 7% villous and 26 6% smooth surface lymphocytes In general the percentage results agree with those of E and EAC binding lymphocytes of human tonsils as previously reported Our serial experiments using immunological techniques proved the existence of two types of lymphocytes (T and B cell) having immunological roles

In our recent investigation of the immunological roles of human tonsil lymphocytes, it was proved that the lymphocytes of tonsils can be differentiated into two subpopulations—bone marrow derived (B) and thymus-dependent (T) cells—which have characteristic membrane markers rosetting with sheep red cell (Tabata et al 1974) Further investigation, using an immunofluorescent technique revealed that human tonsils contain a certain type of lymphocyte (B cell) with immunoglobulin on their surfaces (Tabata et al , 1974) The present study is an attempt to characterize the surface topology of human tonsil lymphocytes by using the scanning electron microscope

MATERIALS AND METHODS

Experimental materials were obtained from patients with hypertrophied tonsils due to the recurrence of chronic inflammation The patients were otherwise free of systemic disease or complicating factors

Lymphocyte preparation

The preparation of lymphocytic suspensions of human hypertrophied tonsils using Angio Conray Ficoll was performed according to our previous report (Tabata et al , 1973) The cells of approximate 10^7 were put on a silver membrane with 0 8 µm porosity (Flotronics Spring House, Pa) by aspiration The silver membranes with the harvested cells were fixed at least half an hour in pH 7 3 1% glutaraldehyde and rinsed twice with pH 7. phosphate buffer Subsequently, post fixation was carried out in 1% osmium tetraoxide 1 hour and rinsed again in the buffer Dehydration was performed in a graded series of alcohol for 5 min each The silver membrane with fixed and dehydrated monolayered cells was prepared for critical point drying in carbon dioxide Critical point drying of the specimen was performed as described by Pollak et al (1973) The portions of the silver membrane were then coated with a thin layer of carbon and gold on a rotating stage

Observation of specimens

JSM-35 scanning electron microscope was operated at an accelerating voltage of 30 kV

RESULTS

The clinical conditions and the percentages of cell types of each sample were summarized in Table 1 An actual counting of 150 cells from

ab I *A su nmari ed result of the percentages of smooth and villous cells*

ase	Sex	Age	Cl n cal d agnos s	Smooth cells (T) (%)	V llous cells (B) (%)	Ind stingu sh able cells (%)
T S	male	13	Hypertroph c tons l	31 4	33 3	35 3
M	male	34	Hypertroph c tons l	24 3	47 2	28 5
K	male	10	Hypertroph c tons l	[illegible] 1	41 6	34 3
Mean				26 6	40 7	32 7

EM m crographs was performed from each ample Fig 1 is a general view of the mo olayered tonsil lymphocytes under SEM s can be seen from the Table from 3 3-47 2% of the tonsil lymphocytes had ir gular surface architecture demonstrating otrus ons (microvilli) with various lengths g 2) Fa rly smooth cells without protru ons on the surface on the other hand were so observed (Fig 3) The percentage of ese smooth cells ranged from 24 1 31 4% It a fact that there are indistinguishable cells om the identical two types of cells having ss or shortened protrusions on the cell sur ces (Fig 4)

DISCUSSION

In 1974 Tabata et al reported that subpopula tions of the lymphocytes from human tonsils were capable of rosette formation with SRC and SRC sensitized with anti SRC antibody complement It was accepted that human tonsils may well supply two different kinds of lymphocytes that is bone marrow derived (B) and thymus dependent (T) cells and which produce antibody with their collaboration

Recently scann ng electron microscopy has been used to reveal the surface architecture of lymphocytes

In 1973 Polliak et al announced that distin guishing between normal B and T lymphocytes

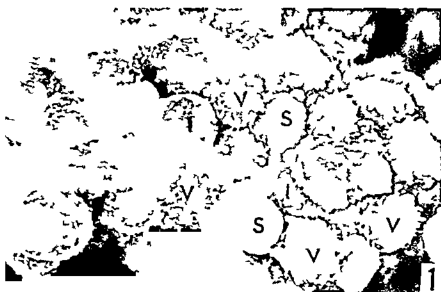


Fig 1 A low magn f ca on of monolaye ed tons l lymphocytes ×3000 V Villous ce (B ce equ valent) S Smoo h e l (T cel equ valen) I Ind s ngu shab e cell



Fig 2 Typical villous cells ×10000

of human peripheral blood (PBL) was relatively easy, in most instances on the basis of their surface architecture under SEM It was con firmed that 20% of the lymphocytes of PBL had villous surfaces identical with B cells, while the majority of T cells (80%) were relatively smooth and smaller in size

Our present study on human tonsil lympho cytes revealed that the proportion of th lymphocytes with villous surfaces reache 40 7% on the average Contrary to th percentage of villous lymphocytes 26 6% wa the average of the smooth lymphocytes It i assumed that a large proportion of T cells fror



Fig 3 Smooth cells without d stinct p n 10000



f Indistinguishable cell having fewer and shorter ısions Adjacent villous cell ×10 000

report by Polliak and his co-workers are butable to involvement of the relative oth cells (40%) with shorter microvilli In experiment, however, these cells were sified into indistinguishable cell groups be- e of lack of evidence of distinction

is of interest however, that the proportion illous and smooth cells by SEM topology ords with that of E (22 6%) and EAC 7%) binding lymphocytes of human tonsils reviously reported by Tabata et al , 1974 It well known, furthermore, that B cells in ans and mice have easily demonstrable unoglobulins on their surface which in ct with antigen Recently, a membrane im nofluorescence technique for detection of B phocytes in human tonsils (Tabata et al 4) proved that on the average approximately 30% (20–35 4%) of the cells had polyvalent immunoglobulins on their surface For easy understanding, the typical cells having surface immunoglobulins are shown in Fig 5 Similar results of the presence of approximately 30% surface immunoglobulin bearing cells in human spleen were reported by Grey et al (1971)

The slight discrepancy in the percentages between surface immunoglobulin bearing cells and villous cells in human tonsil lymphocytes will be resolved when attention is given to discovering the real characteristics of indistin guishable lymphocytes under SEM

ACKNOWLEDGEMENT

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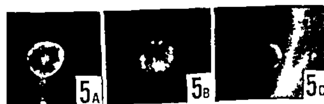


Fig 5 Surface immunoglobulin bearing lymphocytes from human tonsils by membrane immunofluorescence ×400 A ring B patchy C capping

ZUSAMMENFASSUNG

Eine Oberflachen Topologie der in den menschlichen Tonsillen befindlichen Lymphozyten ist mit dem Rasterelektronmikroskop durchgeführt worden Es wurde deutlich, dass die menschlichen Tonsillen aus 40 7% zottigen und 26 6% glatten Lymphozyten bestehen Im allgemeinen stimmen diese Prozentsatze mit denen der E und EAC bindenden Lymphozyten der menschlichen Tonsillen überein Es scheint mir aber, dass noch ununterscheidbare Zellen existieren, wenn auch eine experimentelle Bestatigung fehlt Eine Reihe von Experimenten mit immunologischer Technik bestatigt nun die Existenz der 2 Typen von Lymphozyten (T- und B Zelle), die eine immunologische Rolle spielen

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T Tabata M D
Research Institute for Medical Sciences
Wakayama Medical College
9 Bancho
Wakayama-shi
Japan

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